Ellory, 1974). Transport systems in human red cells studied by this technique include the sodium pump and its partial reactions assayed as Na, K-activated ATPase, K-dependent p-nitrophenylphosphatase (PNPPase) or ouabain-binding, and the nucleoside transport system, assayed by the specific component of nitrobenzylthioinosine binding (Jarvis & Young, 1978). Irradiation of the sodium pump assayed as Na, K-activated ATPase yields a component of molecular weight $330 \pm 30 \times 10^3$ (12) daltons, with both ouabain-binding and K-dependent PNPPase activity inactive with a target size of $180 \pm 28 \times 10^3$ (12) daltons. For the nucleoside transport system, results plotted as log activity *vs.* dose consistently yield biphasic curves, where 30–45% of the binding inactivates with an apparent high molecular weight $> 10^6$ daltons, the remaining 55–70% of the activity giving a target size of $128 \pm 25 \times 10^3$ (5) daltons.

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An apparatus which registers the amplitude of systolic blood flow in a carotido-femoral shunt

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COMMUNICATIONS

Gating of the sodium conductance in the giant axon of the crab *Carcinus maenas*

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Using a voltage-clamp technique similar to that employed by Hille & Campbell (1976) on muscle fibres, the characteristics of the sodium system in single axons (*ca.* 60 μm in diameter) from the nerve of the walking leg of the crab were studied. Fig. 1 shows three examples of records of the sodium (*B*) and the corresponding gating currents (*A* and *C*). The maximum Na-conductance ranged from 64 to 388 mmho/cm^2. The saturating value of Q_{on} ranged from 15·8 to 37·7 nC/cm^2. For 1·5 msec pulses Q_{on} was roughly equal to Q_{off}. Satisfactory least squares fit of the Na-currents were obtained using the equation

$$I_{Na}(t) = I_{Na,\,max}(1 - e^{-t/\tau_m} \cdot e^{\delta})^3 \cdot e^{-t/\tau_h},$$

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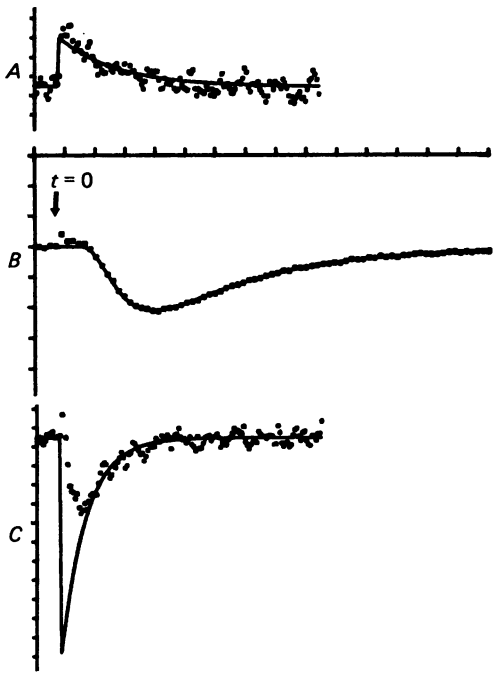

Fig. 1. Average gating and sodium currents from a giant axon of the crab. For records *A* and *C*, the fibre was in Na and K-free saline plus 300 nM-TTX. For record *B*, the fibre was in K-free sea water. Internal solution: 400 mM-CsF, 47 mM-NaF, 50 mM-TEA, 28 mM Tris Cl at pH 7·3. Ordinates: *A*, *C*, 70 μA/cm^2 per subdivision and, *B*, 3·3 mA/cm^2. Time axis: 80 μsec per subdivision. Holding potentials: -116 mV for *A* and *C*; -96 mV for *B*. Sampling interval: 5 μsec for *A* and *C*; 15 μsec for *B*. Temperature 14·5 °C. Solid lines represent the least squares fit to the points. Calculated parameters as follows: *A*, $\tau_{on} = 149$ μsec; $Q_{on} = 22{\cdot}6$ nC/cm^2; *B*, $\tau_m = 76$ μsec; $\tau_h = 290$ μsec; $\delta = 53$ μsec; *C*, $\tau_{off} = 64{\cdot}2$ μsec; $Q_{off} = 31{\cdot}0$ nC/cm^2.

where δ represents a delay in the start of the Na-conductance. The average coefficient of determination of the fit was 0·98.

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Blood alanine as a regulator of amino acid release from muscle in rats

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We studied earlier the uptake or release of individual amino acids from the skeletal muscles by comparing their concentrations in venous blood leaving the muscles with those in arterial blood in anaesthetized rats, showing that the muscles play a major part in regulating blood levels of amino acids and glucose in various circumstances (Daniel, Pratt & Spargo, 1976, 1977*a*, *b*, *c*; Spargo, Pratt & Daniel, 1979). We con-

cluded that a diminution of the arterial levels of the gluconeogenic amino acids was a more potent stimulus than was a diminution in those of the essential amino acids.

We have now examined the effects on the exchange of amino acids between muscle and blood of variations in the blood levels of seven individual gluconeogenic amino acids and find that L-alanine is unique in that it shows a linear relationship between its arterial blood levels and its movement between muscle and blood. (The equation for a line fitted by linear regression is $D = 0{\cdot}272–0{\cdot}394A$, with a coefficient of correlation of 0·94, where D is the venous minus arterial difference of alanine and A is the arterial blood level of this amino acid, m-mole $l.^{-1}$). There is a similar linear relationship between these two variables in mice and rabbits.

We conclude that the concentration of alanine in arterial blood provides a signal for the release of amino acids from the muscles, and this further emphasizes the key role of alanine in the regulation of the metabolism of both glucose and amino acids.

A grant from the National Fund for Research into Crippling Diseases assisted the work.

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Localization of sodium + potassium activated ATPase in the pancreas

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Pancreatic secretion of electrolytes and water can be inhibited by ouabain (Case & Scratcherd, 1974). Accordingly, it can be assumed that the cells, which are involved in electrolyte and water secretion, could be identified by autoradiography with [^{3}H]ouabain.

Cat pancreatic tissue perfused with [^{3}H]ouabain ($0{\cdot}7 \times 10^{-6}$ M) was frozen and sections were prepared for autoradiography (Sterling, 1972).

[^{3}H]ouabain binding sites were found predominantly over the duct cells. Relatively few binding-sites were found over acinar cells. In both cases binding sites were observed corresponding to the basolateral membranes.

It is concluded that the bicarbonate-rich pancreatic juice is secreted mainly by the duct cells. The finding that sodium pump is located on the basolateral membranes excludes a *direct* role in the secretion of electrolytes.

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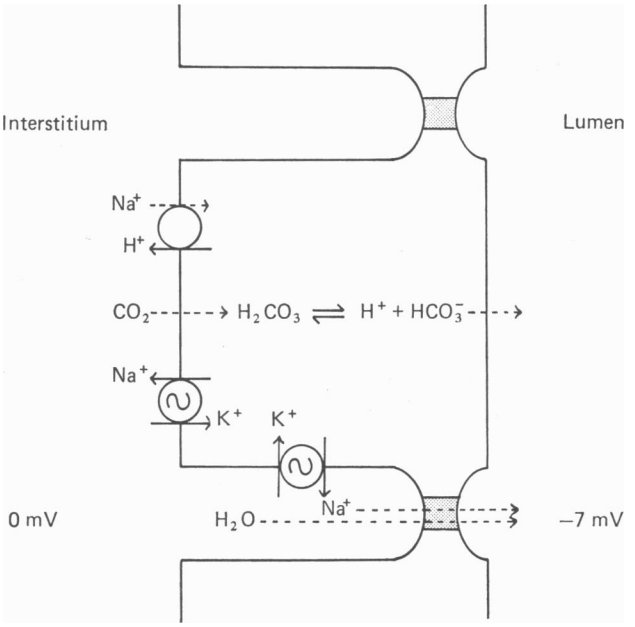

Fig. 1. A model which suggests how the sodium pump could energize the secretion of pancreatic juice. The Na^+-K^+-activated ATPase maintains the Na^+-gradient. The Na^+-H^+-exchange mechanism (Swanson & Solomon, 1972) utilizes the energy of the Na^+-gradient to remove H^+ from the cell. The passive transport of HCO_3^- across the luminal membrane causes the transepithelial potential difference, which acts as the driving force for Na^+. Water follows by osmosis.

Spread of activation along the toad rod outer segment

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We have used the technique of Baylor, Lamb & Yau (1979*a*) to record the membrane current of single toad rod outer segments with a suction pipette. With the outer segment drawn partly into the pipette the sensitivity to a slit of light flashed over the proximal part was at least forty times lower than with the slit over the part within the pipette (see Fig. 1). In other experiments with rods drawn fully into the pipette roughly equal sensitivities were obtained in the two positions. This result shows that internal transmitter released at one point does not diffuse over the entire length of the outer segment. Preliminary estimates set an upper limit of 15 μm for the width at half-height of the longitudinal spread, consistent with the finding of Hagins, Penn & Yoshikami (1970) that in rat rods the action of light is localized to within 12 μm of its site of absorption. A lower limit on the spread can be obtained from the ratio of the single photon response amplitude of 1 pA to the saturating response of 20 pA (Baylor, Lamb & Yau, 1979*b*), which indicates that a length of at least 2·5 μm must be affected.

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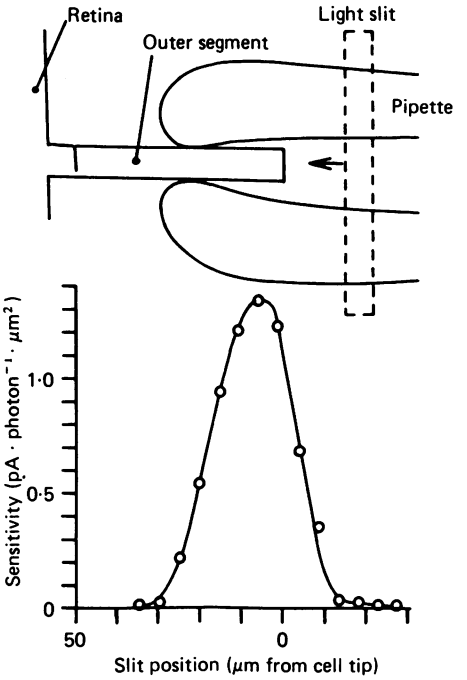

Fig. 1. Sensitivity of a rod outer segment partly sucked into the recording pipette to a slit of 500 nm light flashed at various positions along its length. Saturating response: 7 pA in this position, 26 pA fully sucked in.

Behavioural evidence for the oblique effect in the cat

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In man there is a slight superiority of vertical and horizontal orientations over oblique orientations both in acuity (visual resolution; Campbell, Kulikowski & Levinson, 1966) and in orientation discrimination (Andrews, 1967). Since the discovery by Hubel & Wiesel (1959) that visual cortical neurones in the cat are orientation selective, several attempts have been made to explain the oblique effect in terms of meridional differences in properties of orientation selective cells. An important step before doing so is to demonstrate that the cat has an oblique effect.

In order to demonstrate an oblique effect in the cat we have measured the minimum angular difference (relative orientation threshold) that cats can detect around principal meridians (horizontal and vertical) and around oblique orientations. The discrimination was tested in a go–not go procedure with successive presentations of long black lines on a white background. For each standard orientation (horizontal, vertical, obliques) the cat was trained with progressively smaller angular differences. Training was complete when in three repeated sequences the animal failed to reach the 90% correct criterion for a given angular difference but reached the criterion with the angular difference just larger. After completion of the training the threshold was measured in five sessions with the method of constant stimuli. Thresholds for principal meridians were measured before and after measurement of the threshold

for oblique orientations to exclude order effects. The orientation threshold for principal meridians ranged between 1·5 and 5°. The threshold for oblique orientations was 50–100% larger.

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Direct measurements of image quality in the kitten's eye

BY ANDREW DERRINGTON*. *The Physiological Laboratory, Cambridge*

Measurements of spatial contrast sensitivity show that cells in the kitten's striate cortex are less sensitive and have poorer spatial resolution than their adult counterparts (Derrington, 1978). However it is not known whether the difference can be attributed to the poor quality of the kitten's visual optics.

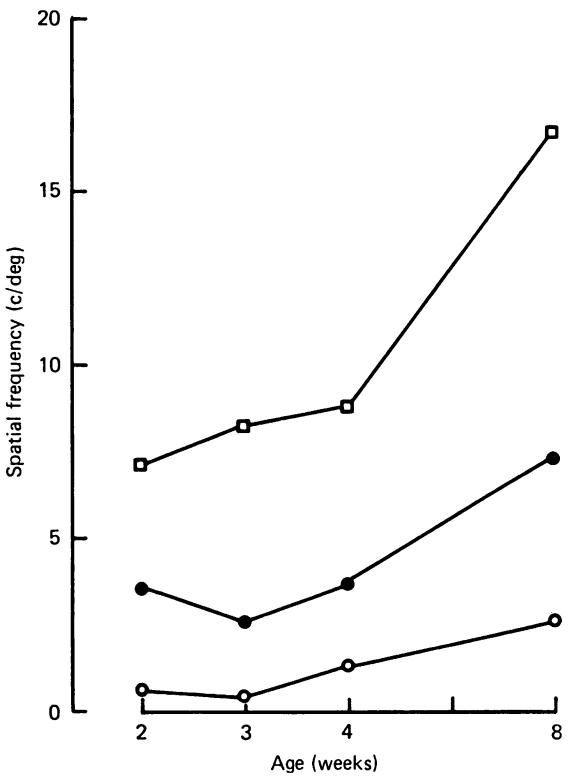

Fig. 1. Spatial frequencies at which MTFs fell to 0·5 (open circles), 0·1 (filled circles) and 0·01 (squares), plotted as function of age.

In order to answer this question an intra-ocular light-guide was used to make direct measurements of the distribution of light in the retinal image of a straight lamp filament, using a procedure like that described by Enroth-Cugell & Robson

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(1978). Measurements were made in four cats, aged between 2 and 8 weeks, paralysed with Flaxedil and anaesthetized with Nembutal. A contact lens with a 3 mm artificial pupil was used.

Modulation-transfer functions (MTFs) were calculated from the illuminance profiles. Fig. 1 shows the spatial frequencies at which the MTF fell to 0·5, 0·1 and 0·01 at different ages. The MTF was also used to calculate the effect of the poor optical quality of the kitten's eye on measurements of sensitivity and spatial resolution. Although the effects were significant they were small in comparison with the observed developmental changes.

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Balance between pattern and movement channels in human vision

BY J. J. KULIKOWSKI and T. R. VIDYASAGAR. *Visual Sciences Laboratories, Department of Ophthalmic Optics, U.M.I.S.T., P.O. Box 88, Manchester M60 1QD, England*

When a sinusoidal grating is alternated in phase or is drifting, separate contrast thresholds can be set for pattern and movement detection (Kulikowski, 1971) due to involvement of different mechanisms (Tolhurst, 1973), one depending on contrast, the other on a transient change in contrast (Kulikowski & Tolhurst, 1973).

It was suggested that, although these detection mechanisms are separable, they interact in producing a percept in response to visual stimulation (Kulikowski, 1975). The ratio of pattern/movement thresholds roughly indicates relative mobility: coarse gratings alternated in phase or drifting seem to move fast not only near threshold but also when their pattern threshold is exceeded, whereas finer gratings seem slower. At a moderate alternation rate (2 Hz) the maximum resolvable spatial frequency for movement detection is about half that for pattern detection; this holds for both foveal and peripheral viewing, at which resolution is lower. Between these resolution limits gratings seem static when alternated, or moving slowly when drifting, but never when moving fast (non-transient movement). Thus a medium grating (10 c/deg) is seen foveally as moving fast or peripherally as static since the resolution limits are lower for both pattern and movement in the periphery.

Both pattern and movement threshold can be elevated by adaptation to the same or another spatial frequency. This masking after-effect:

(*a*) occurs over a range of spatial frequencies which is wider for movement than for pattern detection (Tolhurst, 1973),

(*b*) is asymmetrical: pattern detection is masked more effectively by finer than coarser gratings, whereas the movement threshold is more elevated by coarser gratings. Not only can either pattern or movement thresholds be selectively elevated in such experiments, but the suprathreshold appearance of alternating gratings correspondingly changes, namely their degree of mobility indicated by the ratio of pattern/movement thresholds.

Moreover, adaptation to a steady grating not only elevates the pattern threshold but may slightly lower the movement threshold (J. J. Kulikowski & D. J. Tolhurst,

unpublished). Even stronger effects were obtained after adaptation to alternating coarse gratings (e.g. 0·4 c/deg); the alternating test grating (e.g. 1·6 c/deg) looks stationary as its movement threshold is slightly elevated and pattern threshold reduced (disinhibition).

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Competition among hexoses for hepatocellular uptake in the perfused liver of rat

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Evidence for membrane mediation of hexose transport between blood and liver cells has been provided by Williams, Exton, Park & Regen (1968), Goresky & Bach (1970), and by Hooper & Short (1975, 1977), using various preparations. We have now investigated mutual competitions amongst D-glucose, D-galactose, and D-fructose using a double indicator dilution method in the rat perfused liver. This has features in common with the paired tracer method of Yudilevich *et al.* (1979). The perfusion procedure resembled that of Hems, Ross, Berry & Krebs (1966).

TABLE 1. The single pass extraction (E %) of D-glucose, D-galactose and D-fructose and proportionate change (% ΔE) from control induced by 25 mM competing hexose. Each value is computed from the means of four measurements in each of four livers.

	Transported sugar					
	Glucose		Galactose		Fructose	
Competing sugar	E % ± S.E.M.	% ΔE ± S.E.M.	E % ± S.E.M.	% ΔE ± S.E.M.	E % ± S.E.M.	% ΔE ± S.E.M.
Control	49·58 ± 0·56	—	45·35 ± 0·79	—	27·25 ± 0·20	—
Sucrose 1	48·82 ± 0·32	−1·5 ± 0·53	45·16 ± 0·70	−0·03 ± 0·50	26·66 ± 0·27	−2·1 ± 0·28
Glucose	42·75 ± 1·1	−13·76 ± 1·00	36·92 ± 0·86	−18·5 ± 0·35 *	25·37 ± 0·20	−6·8 ± 0·21
Galactose	43·90 ± 0·94	−11·4 ± 0·90 *	33·08 ± 0·56	−27·0 ± 0·45 *	25·4 ± 0·09	−6·7 ± 0·27
Fructose	28·12 ± 0·55	−21·8 ± 0·73 *	41·26 ± 0·58	−9·0 ± 0·41 *	24·86 ± 0·18	−8·7 ± 0·09
Sucrose 2	46·19 ± 0·50	−6·8 ± 0·51	45·38 ± 1·2	+0·006 ± 0·20	26·16 ± 0·05	−4·1 ± 0·04

* Difference significant at the 0·1 % level (paired t test).

Table 1 shows the percentage diminution in first pass extraction of the hexoses in the presence of 25 mM competing sugar. These results are consistent with the hypothesis that the D-glucose carrier population has two components, on one

of which fructose is a better competitor, and on the other galactose is a better competitor.

We are grateful to Ahmadu Bello University, Zaria, for a study fellowship for J.O.I.

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Immunocytochemical identification of gastrin cells in the alimentary tract of the sheep

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In 1964 Gregory & Tracy isolated gastrin from the antral mucosa of the porcine stomach and the availability of highly purified gastrin permitted several groups to raise antibodies against this hormone. Specific antisera have been used to identify, by immunocytochemical techniques, gastrin-containing cells in several species.

Jury & McLeay (1977) reported the presence of gastrin-like activity (assayed biologically) in crude extracts made from whole tissue of the forestomachs, abomasum and proximal small intestine of the sheep. In view of these observations, we have investigated the distribution of gastrin cells in the alimentary tract of the sheep. Five sheep were killed by I.V. injection of pentobarbitone sodium and perfused with saline followed by 10% neutral buffered formalin solution. Pieces of tissue from defined regions of the alimentary tract were placed in Bouin's fluid for 12–24 hr secondary fixation and then embedded in paraffin wax. Sections were cut at 6 μM and treated by the indirect fluorescence method of Weinstein & Lechago (1977) to localize cells with gastrin immunoreactivity (GIR). Dewaxed, haematoxylin-stained sections were washed in phosphate buffered saline (PBS) and incubated with a C-terminal specific gastrin antiserum (rabbit) diluted to 1:200 with bovine serum albumin (2%) in PBS for 30 min at 27 °C. Sections were washed with PBS and incubated with fluorescein-conjugated goat antirabbit IgG (1:10 dilution in PBS) for 15 min at 27 °C. After rinsing with PBS, sections were mounted in phosphate buffered glycerol for examination under the fluorescence microscope.

Cells containing GIR were most numerous in the mucosa of the abomasal antrum. They were distributed through the entire width of the mucosa and had a characteristic pyramidal shape with a narrow apical pole projecting into the lumen of the pyloric glands. Specifically staining cells were sparsely scattered in the villi and crypts of the duodenum and proximal jejunum and some were located in the submucosal Brunner's glands. It is probable that many of the stained cells in the small intestine contain

* A.R.C. Scholar.

cholecystokinin which would cross-react with C-terminal specific gastrin antiserum. Cells or nerves containing GIR were not found in the oesophagus, the forestomachs, abomasal fundus, ileum, large intestine and pancreas. The addition of excess gastrin to the antiserum abolished the histochemical reaction.

These results suggest that the bioactive gastrin-like material extracted from the forestomachs of sheep by Jury & McLeay (1977) is not gastrin.

Dr G. J. Dockray kindly provided the gastrin antiserum (L48).

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The effects of fasting on the Real kinetic parameters for L-valine absorption measured in jejunum and ileum *in vivo* in fowls

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The effect of nutritional deprivation on the mechanisms of amino acid absorption from the intestine *in vivo* has been poorly characterized. The absorption of ^{14}C L-valine over a range of concentrations (1–10 mM) was measured *in vivo* in the jejunum and ileum of halothane anaesthetized fowls either fed or fasted for 72 hr. The results, calculated per unit surface area of mucosa, were corrected for a linear non-saturating component and subjected to kinetic analysis by computer, and the Apparent K_ms and J_{max}s were calculated. From these data the best estimates of Real K_m and J_{max} corresponding to the Apparent values in the presence of the measured *in vivo* unstirred layer were obtained (Barber, Levin & Mitchell, 1978). The term 'Real' is thus used to designate kinetic parameters of the transport mechanism calculated from the *in vivo* absorption data by the direct linear plot (Eisenthal & Cornish-Bowden, 1974) after correction for non-saturation and the influence of the unstirred layer. The influence of fasting on the Real K_m and J_{max} in the jejunum and ileum were compared.

Fasting produced a significant decrease ($P < 0{\cdot}05$) in the Real K_m in the jejunum from $3{\cdot}2 \pm 0{\cdot}7$ mM (Mean ± S.E.M. $n = 6$) in the fed animals to $1{\cdot}3 \pm 0{\cdot}2$ mM ($n = 5$) in the fasted group. The Real J_{max} was increased significantly ($P < 0{\cdot}02$) from $37{\cdot}6 \pm 7{\cdot}0$ p-mole cm^{-2} sec^{-1} ($n = 6$) in the fed animals to $61{\cdot}8 \pm 4{\cdot}2$ p-mole cm^{-2} sec^{-1} in the fasted ($n = 5$). In the ileum, fasting produced a significant increase ($P < 0{\cdot}01$) in the Real K_m from $1{\cdot}4 \pm 0{\cdot}2$ mM ($n = 6$) in the fed animals to $2{\cdot}3 \pm 0{\cdot}2$ mM ($n = 6$) in the fasted group but there was no significant change in the Real J_{max} (fed $82{\cdot}3 \pm 12{\cdot}7$ p-mole cm^{-2} sec^{-1} ($n = 6$), fasted $94{\cdot}0 \pm 13{\cdot}2$ p-mole cm^{-2} sec^{-1} ($n = 6$)).

The results indicate that the jejunum and ileum respond differently to 72 hr fasting. The absorptive capacity of the ileum, per unit surface area, remains unaltered but there is a possible decrease in the affinity of its transport mechanism for L-valine. The jejunum however, shows an enhanced absorptive capacity per unit

surface area together with a possible increase in the affinity of its transport mechanism for the amino acid.

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Acid responses to gastric distension in the acute rat as an index of intramural plexus activity

BY D. R. KIRK and B. SCHOFIELD. *Division of Medical Physiology, University of Calgary, Alberta, Canada*

Inhibition by atropine of gastrin induced acid secretion in intact whole animal models and chronic pouch preparations is believed to depend on elimination of cholinergic potentiation from the intramural plexuses. Its absence from acute surgically prepared whole animals has not yet been explained. Schofield, Tepperman & Davison (1975) showed that the effect did not depend on anaesthesia alone, but appeared to be a consequence of acute surgical interference. Kowaleski & Schofield (1979) established that the atropine inhibition was present in totally isolated blood-perfused whole dog stomachs. This is compatible with the hypothesis that in the whole animal the cholinergic intramural plexuses are generally depressed by its homeostatic reactions to acute surgery.

The acid responses to distension of the stomach depend on a complex interaction of long vagal and short intramural reflexes. After vagotomy only the latter component is available. The above hypothesis can thus be tested by investigation of distension responses in an acute surgical preparation. A modified Ghosh–Schild (1958) rat preparation was chosen since it is cheap, has been widely used, and the atropine effects under consideration have been shown to occur in this animal.

The general outline of the Ghosh–Schild procedure has been followed. The stomach, however, was perfused with 0·9% NaCl and acid output estimated directly by electrometric titration of the outflow over 10 min periods. During a control period of at least 1 hr the output pressure was set at 2 cmH_2O and distension was applied by raising this to 16 cm for five periods. Accommodation of the stomach occurred during the first period of raised pressure and extra fluid was perfused on the input side to maintain steady output volume. This excess was returned during the two periods following reduction of pressure. Acid output above control level was calculated over six periods, excluding the initial period of accommodation but including the two post-distension periods in which excess fluid was returned, and expressed in μequiv/hr. Vagotomy was then performed in the neck, and after further control periods the stimulus repeated.

Mean results from ten experiments are as follows. Vagus intact $15{\cdot}7 \pm 2{\cdot}5$ μequiv/hr; after vagotomy $6{\cdot}9 \pm 1{\cdot}9$ μequiv/hr. There is thus a well defined post-vagotomy response between $\frac{1}{3}$ and $\frac{1}{2}$ of that with intact vagi. These results therefore do not

support the hypothesis of general depression of cholinergic intramural plexus activity in this acute surgical preparation.

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Effect of propionate on intracellular and surface pH in rat soleus muscle

BY A. DE HEMPTINNE and R. MARRANNES. *Laboratory of Normal and Pathological Physiology, University of Gent, Belgium*

In sheep Purkinje fibres, rapid substitution of 20 m-mole/l. Cl^- with 20 m-mole/l. propionate at pH 6·8 causes intracellular acidosis and transient alkalosis at the surface of the cells (Marrannes, de Hemptinne & Leusen, 1979).

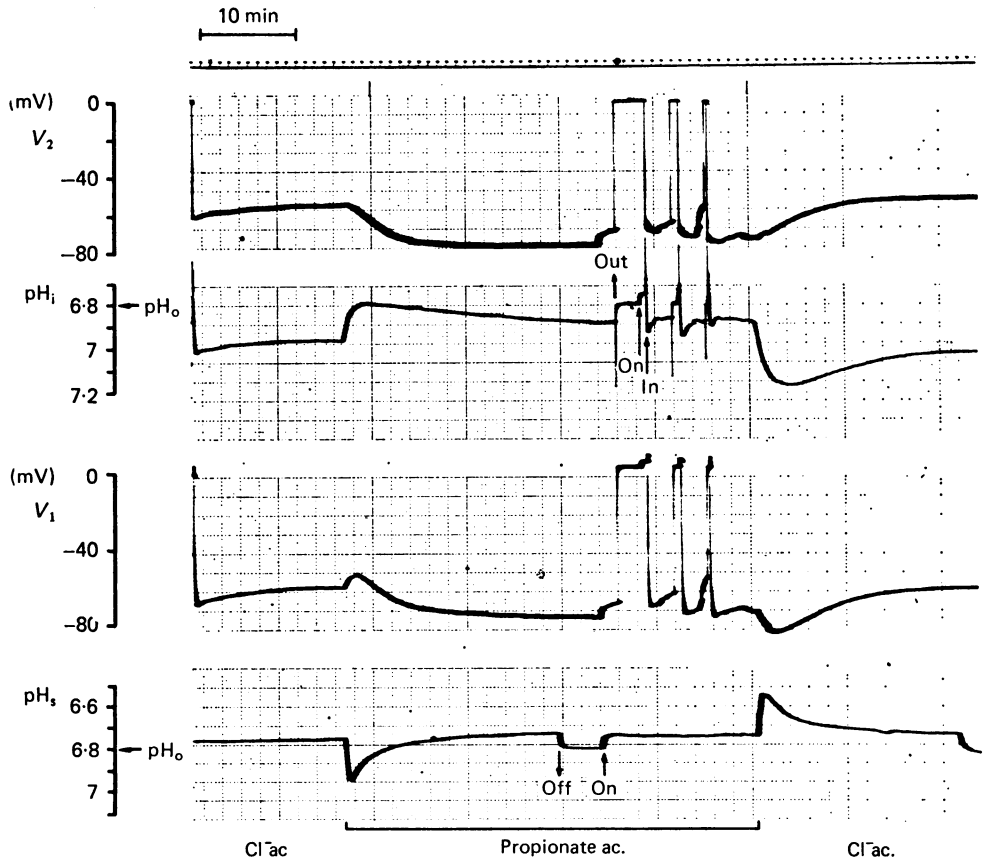

Fig. 1. Influence of propionate (20 m-mole/l.) at pH 6·8 in rat soleus muscle on V_2 transmembrane potential; pH_i intracellular pH obtained as $(V_1 - V_2)$; V_1 potential sensed by pH sensitive electrode barrel; pH_s cell surface pH; pH_o bulk solution pH. The d.b. electrode kept coming out of the cell; it had to be replaced three times. At 'On' the electrode is touching the surface of the cell.

Fig. 1. shows the effects of a similar experiment on rat soleus muscle. Intracellular pH (pH_i) was measured with a double-barrel (d.b.) pH sensitive electrode. Surface pH (pH_s) was measured with an independent micro-electrode which had a rounded tip made of pH glass. Propionate at pH 6·8 causes hyperpolarization, intracellular acidosis and a transient alkalosis at the surface of the muscle cell. The reverse is seen on returning to the control solution. This observation is compatible with the conception that propionate penetrates the cell in protonated form causing intracellular acidosis as it dissociates. In the unstirred layer at the surface of the cell, the unprotonated form remaining in excess acts as a proton sink causing a transient alkalosis.

The shift of pH_i to a more alkaline value in propionate acidosis and the overshoot of pH_i on returning to the control solution suggest the activity of a proton pumping system which opposes intracellular acidosis.

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Calcium, magnesium-ATPase in the microsomal fraction of intima-media smooth muscle from porcine coronary artery

BY F. WUYTACK. *Laboratorium voor Fysiologie, K.U. Leuven, Campus Gasthuisberg B-3000 Leuven, Belgium*

Active Ca transport plays an important role in the relaxation of vascular smooth muscle. The ATP-dependent Ca uptake observed in microsomal fractions of vascular smooth muscle could be considered as a model for studying the Ca transport in these tissues.

In the experiments reported here we demonstrated the existence of a Ca^{2+}, Mg^{2+}-ATPase in the microsomal fraction prepared from porcine coronary artery smooth muscle and we brought forward some arguments indicating that this enzyme activity might be the biochemical correlate of the Ca transport phenomenon. Microsomal fractions were prepared from porcine coronary artery. Ca accumulation was measured by the Millipore filtration technique and ATPase activity by the coupled enzyme assay using pyruvate kinase, lactate dehydrogenase, NADH and phosphoenol pyruvate. Both activities were measured in media in which Ca^{2+} was buffered by means of EGTA. Mg^{2+} was 1·0 mM and solutions also contained 5 mM-Na N_3 to inhibit Ca uptake and ATPase activity of mitochondrial fragments.

The rate of Ca uptake measured in the presence of 5 mM oxalate expressed as a function of the Ca^{2+} was fitted by the Hill equation by means of an iterative non-linear least square method. We obtained a V_{max} of $14{\cdot}57 \pm 2{\cdot}38$ (4) n-mole (mg protein)$^{-1}$ min^{-1}, a K_m of $0{\cdot}34 \pm 0{\cdot}03$ (4) μM and a Hill coefficient (n) of $1{\cdot}69 \pm 0{\cdot}09$ (4) (four experiments including a total of twenty-four observations). The Ca^{2+}, Mg^{2+}-ATPase was measured in the presence of 10^{-4} M ouabain to inhibit the Na^+, K^+-ATPase. The remaining 'basal ATPase' was stimulated by Ca ('extra ATPase'). This 'extra ATPase' was further stimulated by 10^{-5} M of the Ca ionophores A23187 or X537A or by 20 μg/ml. alamethicin. It is believed that the Ca-ionophores prevent an inhibition of the Ca pump because they limit the intravesicular Ca accumulation

by increasing the Ca permeability of the vesicles. Alamethicin would form larger and less selective pores allowing also ATP to enter the vesicles.

It was found that the Ca^{2+}, Mg^{2+}-ATPase measured in the presence of these ionophores had a K_m value for Ca of $1{\cdot}17 \pm 0{\cdot}15$ μM (6) and a Hill coefficient of $n = 1{\cdot}23 \pm 0{\cdot}17$ (6) (six experiments including seventy observations).

We were therefore able to demonstrate in vascular smooth muscle a Ca^{2+}, Mg^{2+}-ATPase activity which we believe is related to the Ca transport for the following reasons:

(1) The ATPase as well as the Ca transport both have K_m values in the μM range.

(2) The Ca^{2+}, Mg^{2+}-ATPase is stimulated by ionophores by preventing net Ca accumulation.

(3) When measured under V_{max} conditions the rates of net Ca transport (in the presence of 5 mM oxalate) and of the 'extra ATPase' activity (in the absence of ionophores) have similar values (Ca/ATP = 0·73).

The effect of magnesium-ATP concentration on the mechanical response of insect fibrillar flight muscle

By M. G. A. Wilson. *Department of Biology, University of York, Heslington, York YO1 5DD*

The effect of substrate concentration upon the mechanical properties of the dorsal longitudinal muscle of *Lethocerus cordofanus* has been investigated. Small (peak-to-peak strain 0·001) sinusoidal length changes of various frequencies were applied to bundles of glycerinated fibres, which were bathed in solutions containing Mg-ATP at concentrations of 0·1, 1 and 10 mM. Complex stiffness measurements were made using a Resolved Component Indicator (Solartron model VP 253.3) in the range 0·5–100 Hz (Fig. 1).

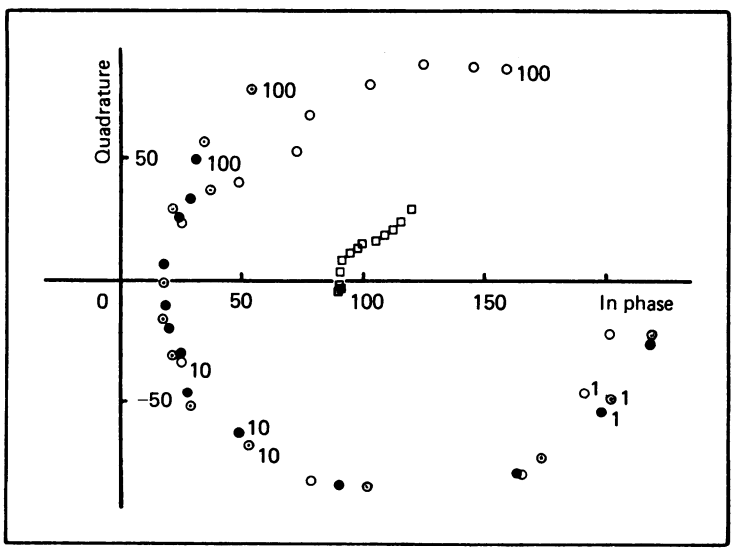

Fig. 1. Complex stiffness as a function of frequency at three Mg-ATP concentrations. ○, 0·1 mM; ⊙, 1 mM; ●, 10 mM; □, relaxed. Four fibres, 12·5 °C, pCa 4·3. Units on axes, μN/fibre per % length change. 1, 10 and 100 Hz points at each substrate concentration indicated.

Points on the figure obtained at the highest frequencies were markedly shifted anticlockwise as substrate concentration increased, without greatly affecting the shape of the plot. This effect could arise if a rapid mechanical process in the preparation increased with substrate concentration in the range 0·1–10 mM. By analogy with the actomyosin ATPase cycle (Lymn & Taylor, 1971) the most likely mechanical process so affected would be detachment of the myosin cross-bridge from the thin-filament site.

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A cellular basis for lidocaine's anti-arrhythmic action

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Cardiotonic steroids or potassium depleted solutions decrease Na pump activity and lead to the development of an arrhythmogenic transient inward current, TI (Lederer & Tsien, 1976; Eisner & Lederer, 1979). The TI produces a slow 'transient depolarization' which can reach threshold and thereby initiate extrasystoles.

Although the antiarrhythmic agent diphenylhydantoin can decrease the magnitude of the 'transient depolarization' (Rosen, Danilo, Alonso & Pippenger, 1976), currents

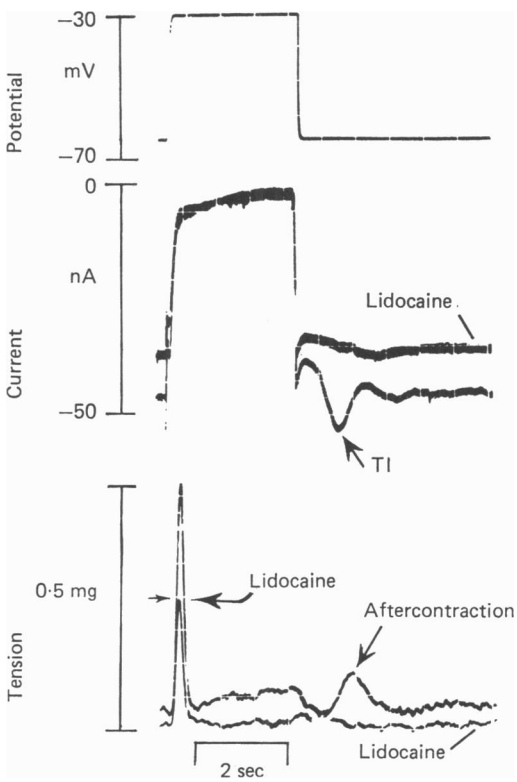

Fig. 1. Effects of lidocaine (35 μM) on membrane current and tension in sheep cardiac Purkinje fibres.

other than the TI may be responsible for this effect. We therefore measured membrane currents under voltage clamp conditions and examined the effects of a more widely used local anaesthetic antiarrhythmic, lidocaine. Fig. 1 shows that the TI produced following a depolarizing clamp step in low (1 mM) K_o is reversed by a high therapeutic concentration of lidocaine (35 μM). Accompanying this reversal is a fall in twitch and tonic tension and removal of the aftercontraction.

This action of lidocaine may be the consequence of a decreased Na entry (local anaesthetic) or may result from a more direct action of lidocaine to modulate Ca_i (see Tsien, Weingart & Kass, 1978).

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Extracellular to intracellular sodium gradient and control of cellular volume in perfused liver

BY L. LAMBOTTE, *Laboratory of Experimental Surgery, UCL 5570, B 1200 Brussels*

There are very few data on the regulation of cellular volume in intact mammalian organs. In this work, the effect of variation of sodium gradient between the extra- and intracellular fluids have been studied in isolated canine liver perfused with Krebs-bicarbonate solution at 20 °C. In this method of perfusion, it has already been shown that membrane potential, intracellular ion concentrations and tissue architecture remain basically normal (Lambotte, 1977).

Administration of ouabain (10^{-4} M) produced an increase in $[Na]_i$ ($+66 \pm 3$ m-equiv/l.) (S.E. of mean, $n = 4$), a drop in $[K]_i$ (-53 ± 8 m-equiv/l.), but also a drop in membrane potential and a corresponding elevation in $[Cl]_i$ ($+25 \pm 8$ m-equiv/l.) while the cellular volume remained unchanged. This result would suggest that the cellular volume is not entirely determined by the maintenance of a normal ionic concentration resulting from the action of not-inhibited sodium pump or by the loss of KCl balancing the gain in NaCl.

Variation of $[Na]_o$ or external osmolarity had also been studied. When one third of the extracellular sodium chloride was replaced osmole for osmole by sucrose the cellular volume and intracellular ion concentrations remained unchanged. Without replacement by sucrose the intracellular water content increased to 142% with a reduction of the $[Na]_i$, $[K]_i$ and $[Cl]_i$ corresponding to the penetration of pure water. This swelling decreased progressively so that after 120 min water content was reduced to 118% of control. In the presence of ouabain the initial changes were similar but the secondary shrinkage did not occur. When a reduced extracellular sodium concentration was corrected, the cellular volume returned quickly to its control value but not in the presence of ouabain. In that case the previously achieved elevated volume remained constant.

In conclusion, sodium can be considered as an effective osmotic agent for respiring

hepatocytes in contrast to what has been reported for renal cortical slices by Robinson (1978). However a single pump and leak model can hardly afford to explain all our data. The involvement of some volume controlling mechanism has to be considered. Further it seems that only the cell shrinkage, in contrast to the maintenance of a given volume, is inhibited by ouabain. An alternative hypothesis is that this volume controlling mechanism can be inhibited by ouabain only when the cells are swollen; in other terms, when the membrane has been somewhat stretched.

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Quantum sensitivity of sodium conductance in toad rods

BY M. CAPOVILLA, L. CERVETTO and V. TORRE*. *Laboratory of Neurophysiology, C.N.R., Via S. Zeno 49, Pisa, Italy*

It is generally accepted that the photoresponse of rods is initiated by a reduction in the Na^+ conductance of the outer segment (Tomita, 1970). A single absorbed quantum of light is able to produce a detectable voltage signal of 0·5–2 mV in the rods of *Bufo* (Fain, 1975; Cervetto, Pasino & Torre, 1977).

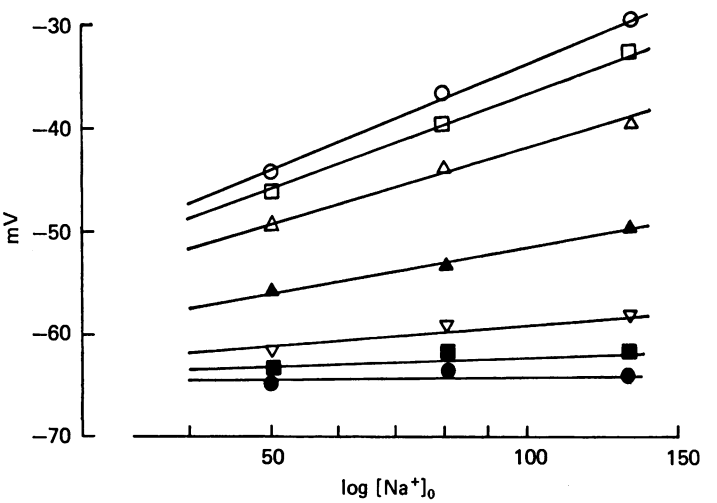

Fig. 1. Relation between maximal voltage response and logarithm of external Na^+ concentration. The slope of the straight line provides a measure of the relative Na^+ conductance. ○, darkness; □, 3·3 Rh*/rod (photoisomerization per rod); △, 13·2 Rh*/rod; ▲, 52·3 Rh*/rod; ▽, 208·2 Rh*/rod; ■, 829 Rh*/rod; ●, 3300 Rh*/rod.

By changing external ionic concentrations it is possible to estimate the relative conductance of various ions both in darkness and during defined conditions of stimulation. It has been estimated that in darkness the relative K^+ conductance is $0·3 \pm 0·05$ and the relative Na^+ conductance is $0·5 \pm 0·1$. At the peak of the response

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to a bright flash the Na^+ conductance is reduced almost to zero and remains so during the initial period of the subsequent plateau.

Fig. 1 shows the light-induced decrease in the relative Na^+ conductance with progressively brighter stimuli. From these data and the quantum voltage sensitivity the decrease in Na^+ conductance induced by a single absorbed photon is estimated to be 7·4 %.

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The effect of helium pressure on guinea-pig isolated tracheal smooth muscle

BY J. MCWHIRTER. *Physiological Laboratory, Admiralty Marine Technology Establishment, Fort Road, Alverstoke, Gosport, Hants.*

Since the description of the phenomenon known as the High Pressure Nervous Syndrome in both man and animals, there has been renewed interest in the effects of high pressure on isolated excitable tissues. Work reviewed by Cattell (1936) revealed that amphibian isolated skeletal muscle develops a spontaneous contracture at pressures greater than 100 bar (990 metres sea water). These contractures were not blocked by D-tubocurarine.

We have investigated the effect of pressures between 13 and 67 bar on the smooth muscle of guinea-pig trachea. Paired tracheal chains were made after the manner of Foster (1960) and maintained in Krebs solution at 37 °C. The partial pressures of O_2 and CO_2 were maintained at 0·95 and 0·05 bar respectively to both chains, the gas being added to increase the pressure on one chain in a specially designed hyperbaric chamber. The responses of both the one bar control and the hyperbaric chain were monitored isotonically.

Electric field stimulation of tracheal chains elicits a biphasic response of a contraction followed by a relaxation. At pressures of 31 and 43 bar the size of the relaxation response to a given frequency is reduced. Furthermore, the time taken to recover from this response is prolonged.

These changes are accompanied by a reduction in the inherent tone of the preparation. This response is variable in magnitude, the mean response increasing with increasing pressure, although these differences are not significant. The tone is steady at the new level within 10 min of compression and is partially reversed on decompression.

Atropine (10^{-6} g/ml.) blocked the contractile response to field stimulation but had no effect on inherent tone. At this concentration atropine increased the mean relaxation observed following compression to 31 and 43 bar. This is not a significant increase however.

Tetrodotoxin (10^{-6} g/ml.) blocked all responses of the smooth muscle to field stimulation but had no effect on inherent tone. At this concentration tetrodotoxin had no effect on the mean magnitude of relaxation following compression but significantly reduced the variation in size of the response.

The evidence suggests that the relaxation response observed on compression to

pressures greater than 13·1 bar is due principally to a direct effect on the smooth muscle.

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Temporal responses of achromatic and colour systems

BY K. KRANDA. *Department of Ophthalmic Optics, U.M.I.S.T., P.O. Box 88, Manchester M60 1QD*

Reaction times for coloured flashes at, or near, threshold, might reflect the temporal characteristics of detection systems. If reaction times are wavelength dependent (cf. Mollon & Krauskopf, 1973) can they be described in terms of 'sustained' and 'transient' responses of detection systems?

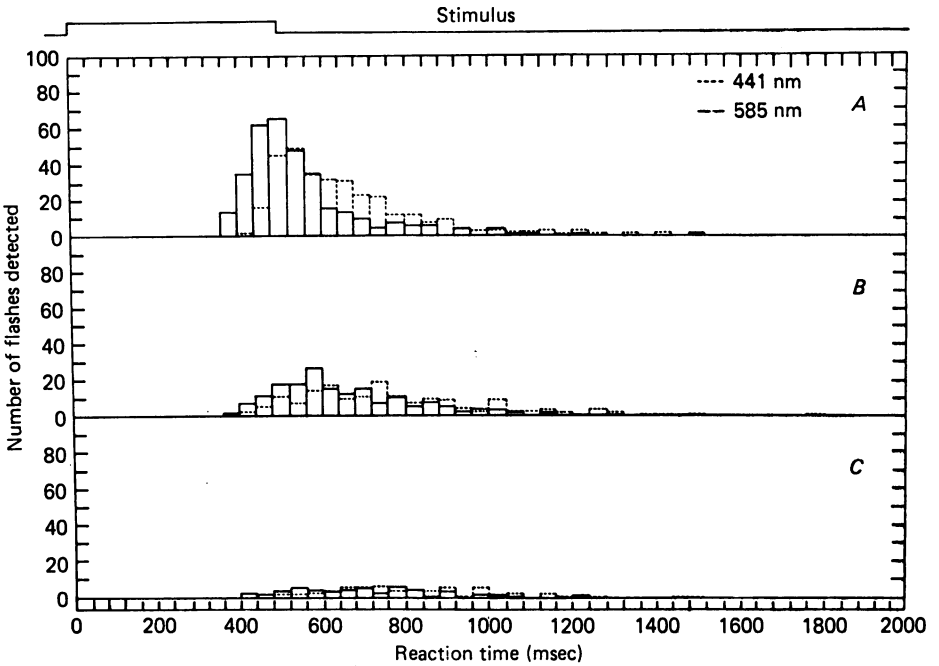

Fig. 1. Reaction time histograms for 1° 0·5 flashes measured at 1·5 $T_I(a)$, $T_I(b)$ and 0·666 $T_I(c)$ levels. n = 375 for each condition.

I measured reaction times for blue (441 nm), yellow (585 nm) and red (676 nm) 1° test flashes on a white background at three threshold intensity (T_I) levels. T_I equals the intensity of a 0·5 sec flash detected with probability of 0·5.

On a 1000 td white background, the achromatic and the colour systems are about equally sensitive to yellow, but only the latter is sensitive enough to detect the blue flashes (cf. Kranda & King-Smith, 1979). If the responses of the achromatic and colour systems were respectively transient and sustained, the reaction time distributions would be sustained as for the blue flashes, but the histograms for the yellow flashes would contain transient and sustained components. The reaction time distributions (Fig. 1) are, however, relatively flat at (*B*) and below (*C*) threshold.

Although the achromatic and colour systems give sustained responses to the 0·5 sec flashes, either system is also capable of responding transiently under different conditions.

I wish to thank W.C.S.M. for support.

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Spatial fibre organization in the optic chiasm of the cat

BY H. AEBERSOLD and U. KUHNT. *MPI Biophys. Chem., D-34 Göttingen, F.R.G.*

It is often assumed in discussions concerning the development of visual pathways that retinotopic order is maintained along the nerve and tract. However, only few experimental data are available from higher vertebrates.

We made twenty-eight penetrations in all regions of the chiasm (track positions were verified histologically) and mapped the receptive fields (RFs) of 361 fibres. Adult cats were anaesthetized with pentobarbitone (35 mg/kg initially, then 1 mg/kg. hr), paralysed with gallamine triethiodide (10 mg/kg.hr) and artificially respirated with a N_2O/O_2 mixture (70/30%). The RF positions were automatically plotted with moving light bars.

A systematic distribution of RF positions was not found in the anterior chiasm. In the posterior chiasm vertical penetrations were clearly associated with RFs either in the upper or in the lower hemifield, not both. Hoyt & Luis (1963) found similarly that the superior–inferior relation in the optic nerve changes to a medial–lateral relation in the tract.

In most penetrations (regardless of the position in the chiasm) the RFs of one eye were situated in the temporal and nasal hemifields whereas the RFs of the other eye lay exclusively in one hemifield; it appears therefore that a few fibres loop into the ipsilateral tract before crossing the chiasm (Hoyt & Luis, 1963).

The RFs of consecutively recorded fibres (lying within 30–80 μm of each other) were often grouped together in a cluster (10–15° diameter). These clusters of RFs were scattered randomly across the visual field (separations up to 50°). Simultaneously recorded fibres always had RFs less than 15° apart. This clustering might reflect the fibre bundles seen in optic nerve sections. However, such fascicles cannot be followed anatomically beyond the anterior chiasm, where they stop abruptly.

In one penetration we recorded from two fibres whose RFs completely overlapped. They came from the same eye but were lying 550 μm apart. This implies that fibres from neighbouring ganglion cells can become separated in the chiasm. This finding is difficult to interpret because the recording site might be located in a region where fibres leave and project to structures other than the lateral geniculate body; i.e. we could be looking at superimposed systems each of which is well ordered. We are attempting to solve this problem by studying fibre organization at progressively higher levels in the optic tract.

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Light adaptation of lateral geniculate nucleus cells in the cat and monkey

By O. D. Creutzfeldt, A. Elepfandt, B. B. Lee and V. Virsu. *Max Planck Institute for Biophysical Chemistry, D-3400 Goettingen, F.R.G.*

Brightness constancy, the Weber law of contrast thresholds, and similar phenomena related to light adaptation would have a simple physiological correlate if the response functions of visual cells were able to shift so as to adequately compensate for changes in overall luminance level. This adaptive compensation occurs in the receptors of several species (Boynton & Whitten, 1970; Normann & Werblin, 1974; Kleinschmidt & Dowling, 1975) and has been found in cat retinal ganglion cells (Sakmann & Creutzfeldt, 1969). We recorded from LGN cells in the anaesthetized cat and macaque (halothane and nitrous oxide during surgery; thereafter 1·5 mg/kg per hour pentobarbitone and 70%/30% N_2O/O_2 mixture) and measured the relation between the contrast of a visual stimulus (large in comparison with receptive field size) and response size at a variety of wavelengths. A 6 mm artificial pupil was used and different adaptation levels were produced by placing neutral density filters in front of the animals' eyes.

For cat LGN cells, using 500 nm wavelength stimuli, brightness constancy and the Weber law appeared to hold down to low luminance levels (0·005 cd/m^2, 0·14 photopic trolands), i.e. a stimulus of the same contrast was able to produce the same response. In monkey LGN cells, adaptive compensation was only partial. Even at luminance levels more than three log units above cone threshold, adaptive compensation of foveal or near-foveal monkey LGN cells was incomplete, and became poorer as cone threshold was approached. Rod input, as revealed by the Purkinje shift, was generally much weaker in monkey LGN cells, even those with fields up to 15° in eccentricity, than in cat LGN cells, as would be expected from the cone and rod dominated retinae of the monkey and cat respectively. As a consequence, the contrast sensitivity of monkey cells was markedly inferior to that of cat LGN cells when the adaptation luminance was below about 1 cd/m^2.

The good adaptive compensation which allows cat LGN cells to display brightness constancy and Weber law behaviour is probably based on rods, for when we used red stimuli, to which rods are insensitive, cat LGN cells behaved like monkey LGN cells. At any event, the adaptive capacity of single cells is much more limited in the monkey than in the cat LGN and seems insufficient to explain the psychophysically observed constancies of brightness, contrast and colour.

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Caffeine and the decay of the McCollough effect

By B. O. Amure. *Physiological Laboratory, University of Cambridge*

The orientation-specific chromatic after-effect of McCollough (ME) has been shown to be sensitive to a variety of centrally active agents of which coffee is one (Shute, 1978). Subsequent experiments using known doses of caffeine in tablet form support the above finding, in that the decay rate of the ME, when measured by the match interference method (Shute, 1977), is increased by taking caffeine. Furthermore, it was found that at a constant dose, the time between administration of drug and adaptation affected the decay rate. In some subjects (2 out of 12) administration of caffeine 10 and 20 min before adaptation induced a fall in the initial ME value. At the 20 min interval, some subjects failed to register any ME. Results are shown in Fig. 1.

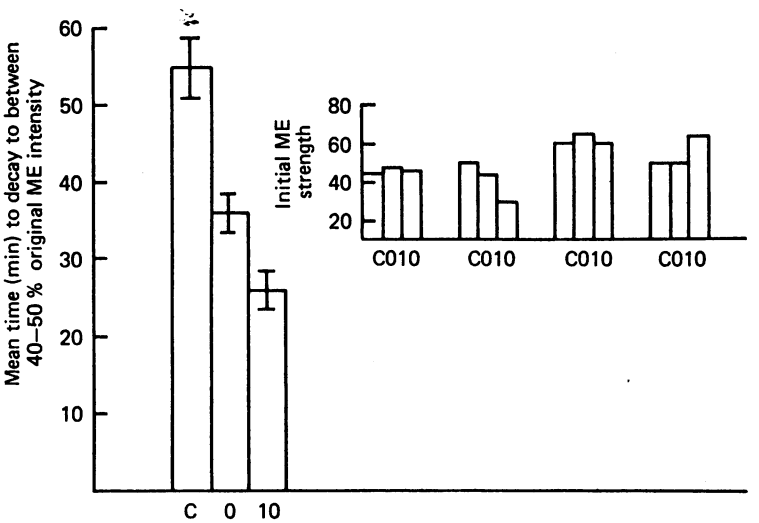

Fig. 1. Comparison of mean times to decay to between 40 and 50 % of original ME intensity (± S.E.M.). Results significant to 95 % level. Inset: effect on initial readings (four subjects). C, control; 0, 0 min; 10, 10 min.

Since anticholinergic drugs have been found to slow ME decay, the effect of caffeine may be due to a depolarizing effect induced by indirect release of acetylcholine into the CNS. The ME appears to be enhanced by certain drugs of which nicotine is one (Amure, 1978).

I wish to thank the M.R.C. for financial assistance.

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Variation in the spatial frequency selectivity of neurones in the cat visual cortex

By I. D. Thompson and D. J. Tolhurst. *Physiological Laboratory, Cambridge CB2 3EG*

Individual neurones in the cat's striate cortex respond to sinusoidal gratings of only a limited range of spatial frequencies. The bandwidth, expressed as the width (in c/deg) at half-amplitude of the frequency tuning curve divided by the optimal spatial frequency, varies markedly between neurones (Movshon, Thompson & Tolhurst, 1978*b*). Is there any significance in this variation?

Single neurone activity in Area 17 was recorded as described previously (Movshon, Thompson & Tolhurst, 1978*a*, *b*). Cats were anaesthetized with 75% nitrous oxide in oxygen supplemented by infusion of pentobarbitone sodium, 0·5 mg/kg.hr, and were paralysed with gallamine triethiode; electroencephalogram and electrocardiogram were continuously monitored. Electrolytic lesions were made during the electrode penetrations to enable a reconstruction of the laminar locations of recorded neurones.

The responses to gratings of different spatial frequencies were measured for neurones whose receptive fields lay within 3° of the *area centralis*. The logarithm of the bandwidth was found to be correlated with the logarithm of the neurone's optimal spatial frequency ($r = -0{\cdot}46$; 232 neurones). The bandwidths of the tuning curves ranged from about 0·25 to 2·5, and for each doubling of the optimal frequency the average bandwidth decreased by a factor of about 0·59. If, in visual cortical neurones, *linear* spatial summation were to occur over a relatively constant area despite considerable differences in optimal frequency, an inverse correlation between optimal frequency and bandwidth would be expected. For instance, in a simple cell, the number of excitatory and inhibitory regions within the receptive field would increase with increasing optimal frequency. Thus, our data may be consistent with the hypothesis that the visual cortex performs a piece-wise frequency analysis of the visual image, abstracting information about several different spatial frequencies within discrete patches of visual field (Robson, 1975).

Of the twenty-nine recorded neurones which had bandwidths of 1·5 or greater, fifteen were recorded either in the deep part of lamina III or the upper part of lamina IVab, where they constituted 29% of the recorded population. In the rest of the cortex, only 8% of the neurones had large bandwidths; this imbalance is statistically significant ($\chi^2 = 14{\cdot}5$ with 1 degree of freedom). It is interesting that this layer is thought to receive W-cell input from the lateral geniculate nucleus (Ferster & LeVay, 1978).

Supported by the M.R.C.

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Morphological differentiation of enkephalin-containing neurones in tissue culture

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Immunoreactivity to enkephalin has been reported in dissociated cell cultures of fetal mouse spinal cord (Neale, Barker, Uhl & Snyder, 1978). We have studied the development of enkephalin-containing neurones in cultures of intact tissue explants from embryonic rat spinal cord, using the method of immunofluoresence staining with an antibody to leucine-enkephalin.

After 7 days' culture of embryonic spinal cord tissue (14–17 days gestation) *in vitro*, when most of the neurones had formed processes and many developed a complex geometry, enkephalin fluorescence was observed in round cells which lacked processes (Fig. 1*A*, *B*). After 14 days, short beaded processes were seen to emanate from the fluorescent cells bodies, and after 21 days most were morphologically well differentiated (Fig. 1*C*, *D*). Maturation was complete between 5 and 7 weeks and at

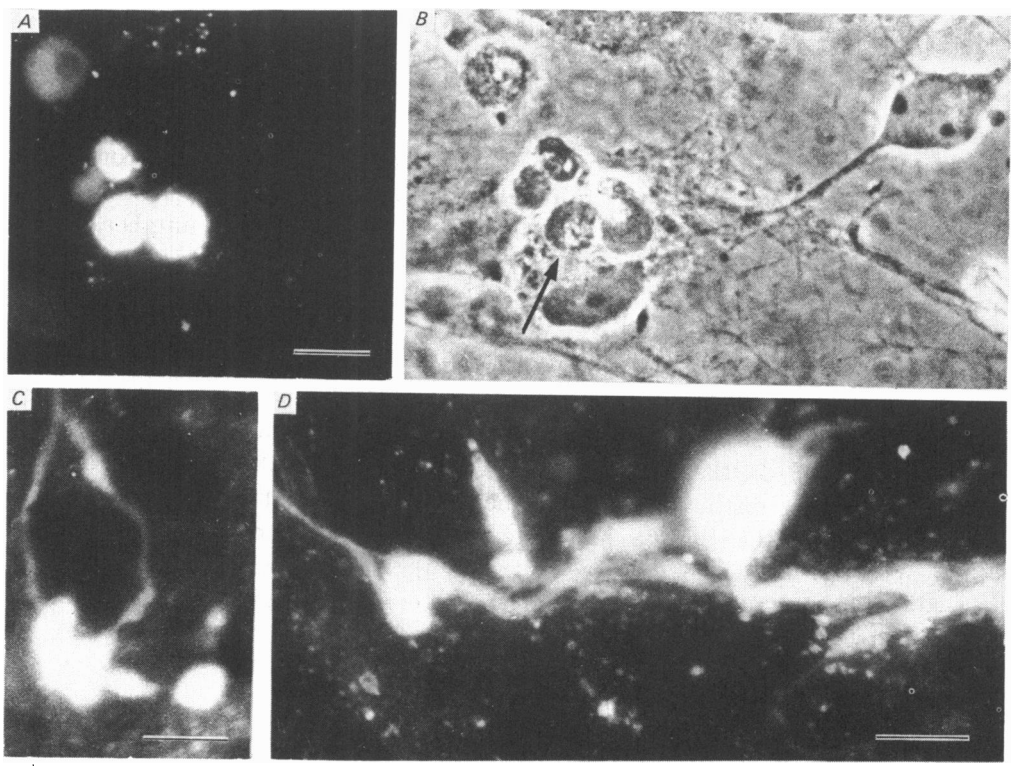

Fig. 1. Developing rat spinal cord neurones reacting to leucine-enkephalin antiserum. *A*, fluoresent cells in 7 days culture. *B*, phase contrast micrograph showing that the cells (in *A*, arrowed) have no processes. Adjacent cells did not fluoresce but had processes. *C*, poorly differentiated neurone in 21 day culture. *D*, well-differentiated neurone in 21 day culture. Bars: 10 μm.

this stage many small cells (10–15 μm perikarya) fluoresced intensely. The micro-anatomical maturation of these fluorescent cells occurs later than the differentiation of most neurones in organotypic cultures and it appears to take place subsequent to the appearance of opiate receptors (Hiller, Simon, Crain & Peterson, 1978)

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The relationship between release of vasoactive intestinal peptide in the salivary gland of the cat in response to parasympathetic stimulation and the atropine resistant vasodilatation

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Vasoactive intestinal peptide (VIP) is present in nerve terminals in mammalian salivary glands (Bloom, Bryant, Polak, Van Noorden & Wharton, 1979) and is a potent vasodilator (Said, 1974). These experiments were designed to discover whether VIP is released from the submaxillary gland, in response to stimulation of the parasympathetic innervation, and can account for the atropine resistant vaso-dilation that occurs in this tissue (Heidenhain, 1872). They were carried out under chloralose anaesthesia and VIP was measured by radioimmunoassay (Mitchell & Bloom, 1978).

At rest the concentration of VIP in the submaxillary venous effluent plasma (38 ± 11 p-mole/l.) was slightly lower than that of the arterial plasma (44 ± 11 p-mole/l.) producing a small positive arterio-venous difference of the peptide. Stimulation of the chorda tympani at 20 Hz for 10 min produced an abrupt rise in the sub-maxillary venous effluent plasma VIP concentration to a peak value of 288 ± 61 p-mole/l. at 5 min and finally 197 ± 9 p-mole/l. when stimulation was discontinued at 10 min. This was accompanied by a threefold increase in submaxillary blood flow that was maintained throughout the period of stimulation. Output of VIP from the gland rose abruptly in response to stimulation reaching a peak of 1150 ± 400 f-mole/min at 5 min and declined rapidly when stimulation was terminated. Closely similar changes in submaxillary blood flow, submaxillary venous plasma VIP concentration and VIP output occurred in response to stimulation of the chorda tympani at the same frequency in atropinized cats (1·0 mg/kg).

Intracarotid infusions of VIP (*ca.* 16 p-mole/min produced a rise in both sub-maxillary blood flow and venous plasma VIP concentration of the same order of magnitude as that observed in response to stimulation of the chorda tympani at 20 Hz. Comparison of changes in salivary blood flow in response to exogenous VIP with those to acetylcholine, when both agents were infused intra-arterially, showed that the salivary vasculature was more than twenty times as sensitive to VIP as to acetylcholine. Intracarotid infusion of acetylcholine (1·4 n-mole/min) had no effect on the concentration of VIP in the salivary venous plasma.

These results provide strong evidence that atropine resistant vasodilation in the submaxillary gland of the cat may be attributable to release of VIP from parasympathetic nerve terminals.

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Long-term effects of tetanic stimulation on blood flow, metabolism and performance of fast skeletal muscles

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Long-term stimulation of fast muscles at a frequency naturally occurring in nerves to slow muscles improves muscle performance and produces changes from predominantly anaerobic to predominantly aerobic metabolism (Pette, Smith, Staudte & Vrbová, 1973; Hudlicka, Brown, Cotter, Smith & Vrobvá, 1977). It might therefore be expected that stimulation at a frequency pattern resembling that occurring in nerves to fast muscles would accentuate anaerobic metabolism. In fact, as shown by the present study, when all motor units are activated, different frequencies of stimulation produce similar changes in muscle metabolism, blood flow and performance.

Blood flow, oxygen and glucose consumption and lactate output were measured during isotonic contractions lasting 90 min in fast muscles (tibialis anterior and extensor digitorum longus) of anesthetized rabbits stimulated via the peroneal nerve with pulses of 0·3 msec duration and supramaximal voltage, either at 10 Hz continuously or with three 5 sec trains/min at 40 Hz. In another series of experiments muscles were stimulated for 2–4 weeks with the same frequency patterns via implanted electrodes as described previously (Brown, Cotter, Hudlicka & Vrbova, 1976), after which similar measurements were made in acute experiments.

After 30 min of isotonic contractions both patterns of stimulation produced equal increases in blood flow, oxygen and glucose consumption and lactate output. Thereafter blood flow, oxygen and glucose consumption remained high in muscles stimulated at 10 Hz and gradually decreased in muscles stimulated at 40 Hz.

In chronically stimulated muscles blood flow and oxygen consumption increased considerably more during contractions than in control muscles, and to the same extent at each frequency of stimulation. Glucose consumption increased similarly in stimulated and control muscle, and lactate output was smaller in muscles stimulated chronically for 28 days at 10 Hz. There were no signs of muscle fibre hypertrophy, and all chronically stimulated muscles fatigued considerably less than controls (to 63–67 % of the peak tension after 14 days' stimulation and 77–87 % after 28 days as compared with 40 % in controls).

In conclusion, stimulation for 14–28 days accentuates aerobic metabolism and improves performance irrespective of its frequency. Total activity may be more

important for the changes in muscle metabolism and performance than the particular frequency of firing.

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Effects of venous pressure elevation on the blood-perfused spleen of the dog

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The time course of the increase in spleen volume resulting from elevation of splenic venous pressure (P_v) (Davies, Richardson & Withrington, 1974) was analysed using techniques designed to distinguish between the responses of the capacitance vessels (Phase-1) and of the splenic microvasculature (Phase-2). In addition, to investigate the mechanism of splenic microvascular changes during P_v elevation, splenic venous haematocrit was measured before, during and after the periods of P_v elevation.

The spleens of anaesthetized dogs were isolated, placed in a plethysmograph and perfused at constant pressure from a cannulated femoral artery. Splenic arterial and venous blood flows and pressures and changes in spleen volume were monitored: the outlet from the splenic venous cannula was arranged to permit control over P_v.

P_v was elevated by 2·5, 5·0, 7·5, 10, 15 and 20 cmH_2O from a control level of +3 cmH_2O, and the rate of increase in spleen volume (dV/dt) during the Phase-2 response measured by three methods: (1) from a point 30 sec after P_v elevation (Wallentin, 1966), (2) by the zero time extrapolation (ZTE) technique (Drake, Gaar & Taylor, 1978), and (3) from a point where the venous blood flow attains a plateau during P_v elevation (Granger, Richardson & Taylor, 1979).

Methods (1) and (2) gave dV/dt values of about 5 and 9 ml. min^{-1} $mmHg^{-1}$ 100 g^{-1} respectively, and over the range 2·5–20 cmH_2O were independent of the P_v increment. Method (3) gave values between about 9·5 and 2·5 ml. min^{-1} $mmHg^{-1}$ 100 g^{-1}, there being an inverse relationship between the increase in P_v and the dV/dt; this could be due either to 'smearing' of the capacitance vessel response at low P_v elevations into the volume increases where dV/dt is measured, or to a protracted vascular response to large P_v elevations increasing the time for venous flow equilibration.

In other tissues, the Phase-2 dV/dt is a volumetric measurement of capillary filtration coefficient and is associated with retention of cell-free fluid. In contrast, in the dog spleen during the Phase-2 volume responses to P_v elevation there is a sequestration of r.b.c.s resulting in reductions in splenic venous haematocrit during the period of P_v elevation, and a subsequent rise in haematocrit above control values as P_v is returned to control levels. Reduction in dV/dt by splenic nerve stimulation (0·5 or 1·0 Hz: Davies *et al.* 1974) is associated with comparable reductions in the changes in splenic venous haematocrit on P_v elevation.

During venous pressure elevation, the dog spleen sequesters r.b.c.s, and the Phase-2 dV/dt response is therefore not comparable to that of other tissues.

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Effects of an inhibitor of cytochrome P_{450} on the responses of the rabbit to arachidonic acid

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Thirty-four rabbits were sensitized to arachidonic acid (AA) by I.V. injection of haemolysed blood, heparin and 8 min of hypoxia (12% O_2) (Deby, Noël, Chapelle, Van Caneghem, Deby-Dupont & Bacq, 1979). The AA50 (dose of AA needed to obtain a 50% decrease in carotid pressure) was lowered by this sensitization to 15–25 μg/kg. Variations in blood flow were registered in a carotid–femoral shunt (Deby, 1979); e.c.g. was continuously monitored and venous pressure measured in the left atria.

Two types of reactions to 30–40 μg AA I.V.:

(1) In nine rabbits, signs of anoxia on the e.c.g. together with arterial hypotension, increased auricular pressure, much reduced systolic flow in shunt. E.c.g. signs did not disappear when blood pressure returned to normal. Death occurred after 60 μg/kg AA.

(2) In twenty-five animals, e.c.g. troubles were seen only when blood pressure fell below 35–40 mmHg and were reversible. Venous pressure was unchanged and systolic flow in shunt increased. The lethal dose of AA was above 250 μg/kg.

After the usual sensitization, five out of six rabbits where the cytochrome P_{450} levels had been at least doubled by 4 days pre-treatment with phenobarbitone presented reactions to AA injections of the first type which were prevented or reversed a few minutes after injection of 20 mg/kg of metyrapone, a potent inhibitor of the processes involving cytochrome P_{450} (Leibman, 1969), one of which is the generation of superoxide anion and hydrogen peroxide (Strobel & Coon, 1971). The fall of carotid pressure after AA injection was not altered by metyrapone, but the e.c.g. signs disappeared, the venous pressure did not increase and the systolic blood flow increased; the lethal dose was very high (600–800 μg/kg). The metyrapone protection lasts at least 5 hr. More experiments are needed to interpret these observations in terms of variations in PGI_2 and thromboxane synthesis induced by AA injections.

Cinti and Feinstein (1976) have shown that metyrapone inhibits the AA-induced platelet aggregation and the formation of aggregating factors from AA by isolated microsomes.

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Insensible heat losses in man during simulated dives in helium–oxygen mixtures to a depth of 420 metres of sea water (43 bar)

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In a hyperbaric helium–oxygen environment comfort temperatures are necessarily higher than normal. The environment often has a high absolute humidity and evaporative heat loss is also attenuated due to the reduction in mass transfer of water vapour from the skin. In addition the high thermal conductivity and density of the surrounding medium means that accidental overheating of the environment may leave a diver ill-equipped to deal with increased body heat storage.

Four subjects were exposed to helium–0·4 bar oxygen at 43 bar while being fed a fixed diet. Daily water intake and water loss in urine and faeces were measured and the combined respiratory and surface evaporative losses were deduced by subtraction. Water losses were $30{\cdot}3 \pm 7{\cdot}9$ g hr^{-1} in air at 1 bar representing about 21 W. Insensible losses decreased with depth to $8{\cdot}5 \pm 9{\cdot}3$ g hr^{-1} at depths between 37 and 43 bar. This represents a fall in evaporative mass flux, due to depth, to approximately 28 % of the 1 bar capability. Serum osmolarity and circulating ADH values remained relatively constant throughout the dive. A feeling of constant wetness is experienced at the skin surface. The phenomenon is reversed with decompression.

An evaporative mass transfer coefficient, h_D, can be calculated from a consideration of the analogy between heat and mass transfer and using the Chilton–Colburn (1934) correction to the Lewis (1927) relation then $h_D = h_c(\mathrm{Pr}/\mathrm{Sc})^{0{\cdot}667}/C_p\,\rho$. Pr and Sc are the Prandtl and Schmidt non-dimensional numbers, h_c = convective transfer coefficient, C_p = specific heat and ρ = density. There is a reduction in the coefficient h_D to 22 % at 200 m (21 bar) and 15 % at 360–420 m (37–43 bar) compared to surface air data. The reason that measured evaporative losses are reduced to only 28 %, when the calculated h_D value diminishes to 15 %, is probably due to the high level of skin wetness increasing the vapour concentration gradient and thus the driving force for evaporation.

The consequences of the reduction in evaporative heat loss capacity are likely to be encountered, particularly in the thermoregulatory response to exercise, and the hypothesis is at least subjectively supported by the heat discomfort felt during relatively short periods of exercise performed at increased depth.

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Regional distribution of pulmonary ventilation and perfusion in the conscious dog

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Analysis of the determinants of regional pulmonary ventilation and perfusion in man is often based on experimental studies in anaesthetized, mechanically ventilated dogs in horizontal or vertically suspended postures. Anaesthesia affects cardiopulmonary function in dogs (Muggenburg & Mauderly, 1974) and effects of posture (Kaneko, Milic-Emili, Dolovich, Dawson & Bates, 1966) and mechanical ventilation (Rehder, Sessler & Rodarte, 1977) on regional lung function in man have been documented. Conscious dogs have been used in studies of pulmonary mechanics (Gillespie & Hyatt, 1974) but not in investigation of regional lung function and the distribution of pulmonary ventilation and blood flow in the normal dog has not yet been established.

In this study, unpremedicated normal greyhound dogs, wearing a specially designed mask, stood in a frame in front of a gamma camera which was linked to a computer. A pneumotachograph allowed measurements of respiratory rate and tidal volume and the electrocardiogram was continuously recorded. Steady state inhalation of the radioactive gas $^{81}Kr^{m}$ (half-life 13 sec, Eγ 190 keV) produced a count distribution image reflecting regional ventilation, while local pulmonary perfusion was assessed during continuous infusion via a jugular catheter of $^{81}Kr^{m}$ dissolved in 5% dextrose. By image division, the distribution of ventilation–perfusion ratios ($\dot{V}_A/\dot{Q}$) in the lung was calculated (Harf, Pratt & Hughes, 1978). Rebreathing the radioactive gas $^{85}Kr^{m}$ (half-life 4·4 hr, Eγ 150 keV) produced an image proportional to the regional distribution of lung volume. Ventilation and perfusion images were divided by the volume image and calibrated with an $^{85}Kr^{m}$ clearance measurement to obtain regional pulmonary ventilation ($\dot{V}_A$) and perfusion ($\dot{Q}$) per unit alveolar volume (V_A) during tidal breathing (Amis, Ciofetta, Clark, Hughes, Jones & Pratt, 1977).

Horizontal and vertical strips were analysed. In five dogs $\dot{V}_A/\dot{Q}$ was evenly distributed in vertical and horizontal directions in the lung. Individual $\dot{V}_A/V_A$ and $\dot{Q}/V_A$ distributions determined in three dogs were also uniform. These findings contrast with significant nonuniformity of function in man in a similar posture (Amis *et al.* 1977) and suggest adaptation to the standing horizontal posture in the dog.

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Effect of propranolol on myocardial oxygen balance during exercise in dogs

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Although beta adrenergic blockade is known to alter both coronary blood flow and myocardial oxygen consumption during exercise, the relationship between oxygen demand and supply of the myocardium in these conditions is not clearly established.

This aspect was investigated in eight conscious dogs chronically instrumented with Doppler ultrasonic flow probes around the left circumflex coronary artery, and with fluid filled silastic catheters in the aorta and coronary sinus. Three to four weeks after surgery, the dogs were subjected to a submaximal exercise on a treadmill before and after propranolol (1–1·5 mg/kg I.V.). Haemodynamic measurements were made at rest and at the third minute of the run.

During control exercise heart rate increased from 109 ± 5 to 206 ± 11 beats/min, mean coronary blood flow rose from 38 ± 5 to 76 ± 9 ml./min, myocardial oxygen consumption shifted from $4{\cdot}55 \pm 0{\cdot}54$ to $10{\cdot}51 \pm 1{\cdot}22$ ml./min, while coronary sinus oxygen content decreased from $4{\cdot}87 \pm 0{\cdot}35$ to $3{\cdot}95 \pm 0{\cdot}24$ vol%. The ratio oxygen delivery/oxygen consumption decreased from $1{\cdot}41 \pm 0{\cdot}04$ to $1{\cdot}29 \pm 0{\cdot}03$. When the same exercise was performed after propranolol, heart rate increased from 99 ± 6 to 160 ± 8 beats/min, mean coronary blood flow from 35 ± 4 to 51 ± 5 ml./min and myocardial oxygen consumption from $4{\cdot}10 \pm 0{\cdot}44$ to $7{\cdot}94 \pm 0{\cdot}89$ ml./min, while coronary sinus oxygen content decreased from $4{\cdot}75 \pm 0{\cdot}57$ to $2{\cdot}58 \pm 0{\cdot}33$ vol%. The ratio oxygen delivery/oxygen consumption decreased from $1{\cdot}40 \pm 0{\cdot}06$ to $1{\cdot}16 \pm 0{\cdot}02$.

The lower values of the ratio oxygen delivery/oxygen consumption and of the coronary sinus oxygen content during exercise after propranolol, indicate that propranolol alters the balance between myocardial oxygen supply and demand during exercise in conscious dogs.

The initiation of inspiration

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It is generally accepted that the activity of pulmonary stretch receptors inhibits inspiration. In artificially ventilated animals this relegates inspiratory efforts to periods of lung deflation. If stretch receptor activity were the only determinant of inspiratory timing, removing this activity should result in a pattern of inspiratory efforts which is identical to that after vagotomy. We have investigated other influences on the timing of inspiration.

New Zealand White rabbits were anaesthetized with 30 mg/kg sodium pentobarbitone. A tracheal cannula was inserted and the pattern of breathing was recorded using a pneumotachograph whose output was electronically integrated to provide a record of tidal volume. The activity of the cut end of a root of the right phrenic

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nerve was simultaneously recorded. The animals were paralysed with gallamine triethiodide (Flaxedil; May & Baker). Pentobarbitone sodium was administered at the same rate as before paralysis to maintain anaesthesia. Ventilation was by intermittent positive pressure in a pattern which closely followed spontaneous breathing. Lung stretch receptors were paralysed by ventilating the animal with 200 p.p.m. SO_2 in air (Callanan, Dixon & Widdicombe, 1975; Davies, Dixon, Callanan, Huszczuk, Widdicombe & Wise, 1978). Abolition of the Hering-Breuer inflation reflex was used as an index of stretch receptor paralysis. The presence of irritant receptor activity was demonstrated by rapid deflations of the lungs which caused an increase in the frequency of the bursts of phrenic discharge (Widdicombe, 1954).

With stretch receptors functioning phrenic discharge invariably occurred during the deflation phase of ventilation. When stretch receptors were blocked phrenic discharge occurred with no set phase relation to ventilation if tidal volume was below resting spontaneous tidal volume. Phrenic discharge was synchronous with the inspiratory and expiratory phases of the pump at higher tidal volumes. Stopping the pump in its deflation phase immediately reduced the frequency of phrenic bursts. Bilateral vagotomy produced a pattern identical with that seen with stretch receptor block and low ventilation volumes.

We have demonstrated a vagally mediated inspiratory initiating effect. Since J-receptors are reported to be inactive under our conditions (Paintal, 1973; Guz & Trenchard, 1971) this probably originates from rapidly adapting lung irritant receptors.

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The effects of hydrostatic pressure on the osmotic fragility of erythrocytes and their protection by pentanol

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High hydrostatic pressure orders lipid bilayers and dissociates protein polymers. We have studied the effects of 1–350 atm pressure on the fragility of human and bovine erythrocytes. The apparatus used enabled blood and hypotonic sodium chloride to be mixed at high pressure after which the cells were fixed before decompression. Haemolysis was then determined spectrophotometrically.

Pressure increased fragility, particularly at 5 °C (Fig. 1*A*). Cells treated with pentanol in the hypotonic solution were also made more fragile by pressure, but in an interesting biphasic manner (Fig. 1*B*).

These data contrast with the limited results of Brewster, Collins, Funnell & Smith

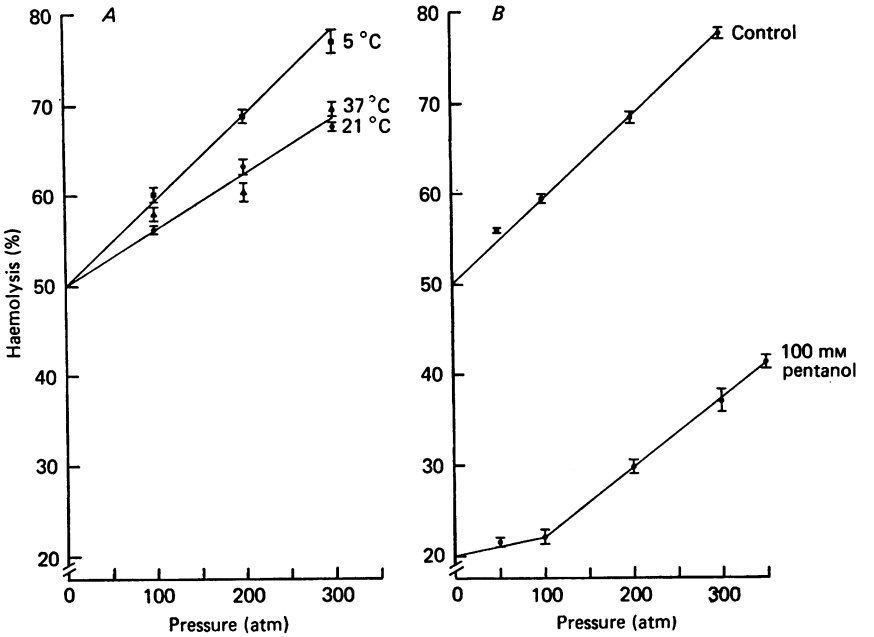

Fig. 1. *A*, human erythrocyte fragility at 5 (■), 21 (●) and 37 °C (▲). *B*, bovine erythrocyte fragility at 5 °C and with 100 mM pentanol.

Results are means ± S.E. (minimum of three experiments). The sodium chloride solution was chosen such that at the experimental temperature it gave 50 ± 10% haemolysis. The haemolysis was then normalized to 50%.

(1976) and leads us to suggest that these effects of pressure are on the protein fabric of the membrane rather than a condensing effect on the bilayer.

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Extracellular calcium control of pancreatic acinar cell membrane conductance during sustained acetylcholine stimulation

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In pancreatic acinar cells elevation of intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ increases membrane conductance while extracellular Ca^{2+} maintains a low resting membrane conductance. Acetylcholine (ACh) increases membrane conductance causing depolarization. This effect seems to be mediated by an increase in $[Ca^{2+}]_i$ (Petersen & Iwatsuki, 1978). Previous experimental protocols employed short-pulse applications of secretagogues. The aim of this work was to investigate the role of extracellular Ca^{2+} in the control of membrane conductance during sustained stimulation.

Simultaneous recordings of membrane potential and resistance from neighbouring

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acinar cells using two intracellular micro-electrodes were carried out on isolated superfused segments of mouse pancreas (Iwatsuki & Petersen, 1977).

Removal of Ca^{2+} from the superfusion solution together with addition of EGTA evoked sustained depolarization and reduction of input resistance. This was similar to the effect of sustained (20 min) ACh (5×10^{-4} M) stimulation in the presence of extracellular Ca^{2+} (2·6 mM). Removal of extracellular Ca^{2+} during ACh exposure caused increase in resistance (about 2·5 MΩ) and hyperpolarization (3·5 mV). In the absence of extracellular Ca^{2+} (Ca^{2+}-free, EGTA 10^{-4} M) sustained ACh stimulation only evoked transient resistance reduction and depolarization. Readmission of Ca^{2+} (2·6 mM) still during ACh stimulation evoked depolarization (about 5 mV) accompanied by decrease of resistance (3·5 MΩ). Mn^{2+} could not mimic this action of Ca^{2+}. Addition of Mn^{2+} (2 mM) to Ca^{2+}-free solution during sustained ACh stimulation caused a slight hyperpolarization (2 mV) with increase in resistance (1 MΩ).

Previous results have shown that potential and resistance changes evoked by short pulses of ACh (1 sec duration) were relatively independent of extracellular Ca^{2+} (Petersen & Iwatsuki, 1978). In contrast sustained resistance reduction and depolarization evoked by continuous superfusion with ACh (over 5–20 min) is entirely and immediately dependent on external Ca^{2+}. Readmission of Ca^{2+} to Ca^{2+}-free solution in the absence of stimulation causes hyperpolarization and increase in resistance while the opposite effect is observed during continuous ACh superfusion. These results indicate that the membrane permeability to Ca^{2+} is enhanced during sustained stimulation. The present results correspond to previous results from studies on Ca^{2+}-dependence of amylase secretion in which it was shown that short pulses of ACh caused short bursts of secretion essentially independent of external Ca^{2+} while external Ca^{2+} is needed in order to maintain sustained amylase secretion during continuous stimulation (Petersen & Iwatsuki, 1978).

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Packeted transmitter release in the mouse vas deferens; an electrophysiological study

BY A. G. H. BLAKELEY* and T. C. CUNNANE. *Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ*

The rising phase of the excitatory junction potential in the guinea-pig vas deferens resolves by differentiation into transient peaks of depolarization which we have named 'discrete events'. Discrete events occurring at constant latencies from the stimuli may represent the transmitter release from single release sites, which when invaded by an action potential release transmitter in a packeted manner. Every action potential does not release transmitter (Blakeley & Cunnane, 1978).

We have now examined transmitter release in the mouse vas deferens. The innervation of the vas was stimulated at 0·2–2 Hz by submaximal field stimulation through

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bipolar platinum ring electrodes around the prostatic end of the vas. Supramaximal stimuli at 0·1 Hz were also used.

Prominent discrete events in the electrically differentiated evoked e.j.p.s were found in 90% of the muscle cells impaled. In these cells large spontaneous e.j.p.s were also found. The amplitude and time course of the discrete event could be matched by spontaneous e.j.p.s in the same cell. At a single latency discrete events occurred to 1 in 1 to 1 in 30 stimuli and their amplitude varied in a step wise manner.

In the mouse, as in the guinea-pig vas deferens, transmitter release is therefore packeted and when the release site is invaded by an action potential a small number of packets are released. Action potentials do not always release transmitter. The incidence of prominent discrete events in the mouse (90% of the cells impaled) is greater than we had found in the guinea-pig (20%). This correlates with the morphological differences between the vasa. In the mouse vas deferens all the cells are innervated (Yamuchi & Burnstock, 1969) compared with only 20% of cells in the guinea-pig (Merrillees, 1968).

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Electrophysiological effects of field stimulation on mouse pancreatic acinar cells

By J. S. Davison and G. T. Pearson. *Physiology Department, Dundee University, Dundee DD1 4HN*

In isolated, superfused segments of mouse pancreas we have shown that electrical field stimulation causes depolarization of acinar cells with an associated fall in membrane resistance (Fig. 1*C*), confirming A. Nishiyama (1979, in the press). This effect is blocked by atropine ($1{\cdot}4 \times 10^{-6}$ M) and is similar to that evoked by vagal stimulation *in vivo* (Davison & Ueda, 1977). The equilibrium potentials for the endogenous transmitter and for acetylcholine (ACh) by ionophoresis were identical (Fig. 1*A*, *B*). Tetrodotoxin (10^{-6} M) abolished the field stimulation response but had no effect on the response to ACh or on spontaneous miniature potentials. Removal of calcium or sodium from the superfusate abolished the response to field stimulation leaving the ACh response unaffected. Miniature potentials were eliminated by calcium removal.

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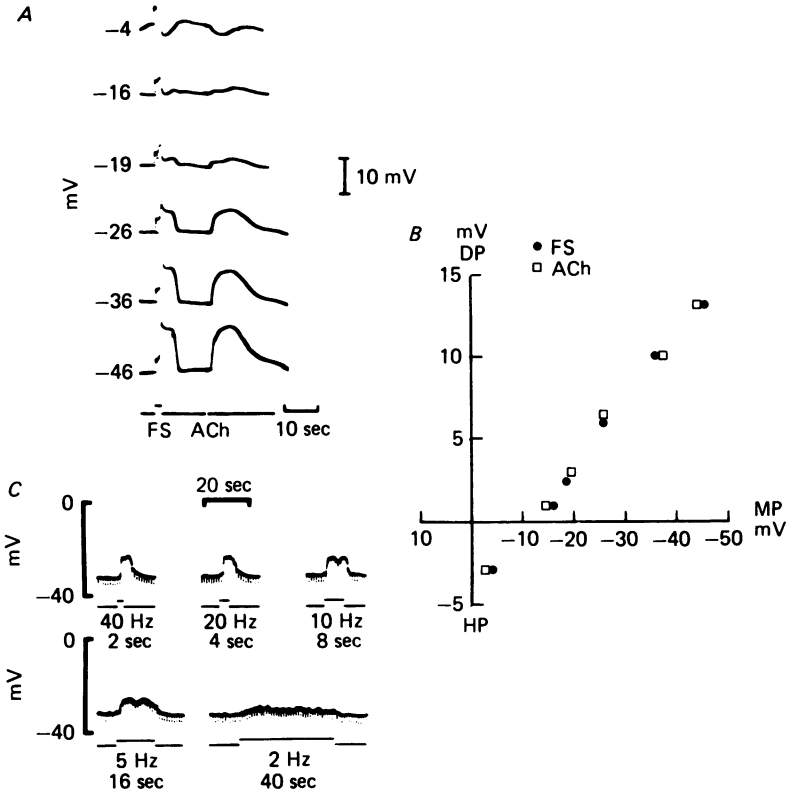

Fig. 1. *A*, field stimulation (FS, 40 Hz, 2 msec, 10 V, 2 sec) and ACh ionophoresis (ACh, 500 msec, 90 nA) effects on one cell at various membrane potentials set by d.c. inject on through the recording electrode. *B*, plot of membrane potential change against resting membrane potential (MP); DP, depolarization; HP, hyperpolarization. *C*, effect of stimulation frequency on membrane potential and resistance. Current pulses (1 nA, 100 msec) are repetitively passed through the recording electrode.

Parotid acinar cells: membrane potential changes induced by electrical field stimulation

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Electrical field stimulation (FS) of isolated segments of mouse parotid evokes changes in acinar membrane potential and input resistance resembling those induced by acetylcholine (ACh) micro-ionophoresis (Fig. 1). The FS response was selectively abolished by tetrodotoxin and by superfusion with sodium-free or calcium-free media, while ACh response persists. FS therefore evokes neural excitation and consequent transmitter release. The equilibrium potential for ACh ionophoresis and FS correspond (Fig. 1). Atropine (10^{-6} M) abolishes both responses.

FS seems a useful technique for investigating the functional innervation of salivary glands. In the parotid the FS response is mediated by ACh, released from parasympathetic nerves.

* M.R.C. Training Fellowship.

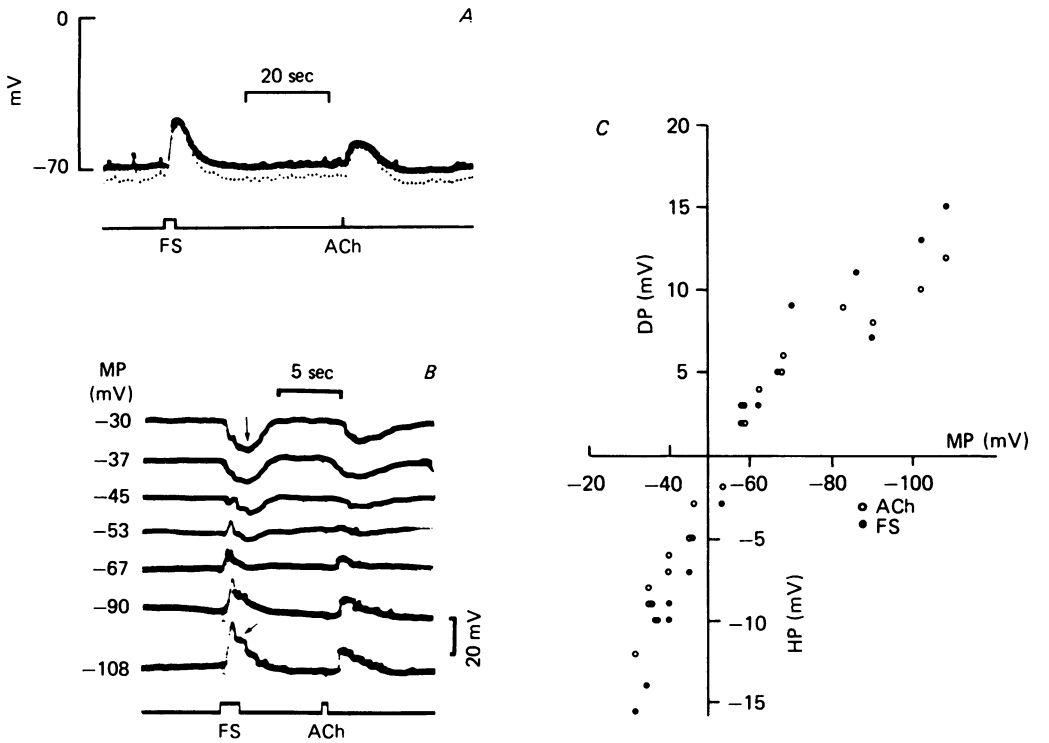

Fig. 1. *A*, change in membrane potential and input resistance (intracellular current pulses 2 nA, 100 msec, 1 Hz) of an acinar cell to supramaximal FS (2 msec, 40 Hz, 15 V, 2 sec) and ACh ionophoresis (10^{-7} A, 500 msec), *B*, potential change evoked by two stimuli in single cell at different levels of resting potential (d.c. application); points of maximum potential change after cessation of stimulation (arrowed) plotted in (*C*) as function of resting potential. MP, membrane potential; HP, hyperpolarizing; DP, depolarizing.

Increased sodium conductance of the apical surface of toad skin treated with aldosterone

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The effects of aldosterone, $\geqslant 0{\cdot}1$ μm, on the abdominal skin of *Bufo marinus* after 15 hr at room temperature in Ringer fluid are shown in Table 1 ($n = 9$ pairs).

The hormone-dependent stimulation of sodium transport appears to be related mainly to a decrease in the ohmic resistance of the apical border of epithelial cells, in other words, to an increase in conductance of that structure for sodium. These results are thus in keeping with the hypothesis (Crabbé, 1977) that aldosterone acts by facilitating the movement of sodium at the apical border of target epithelia.

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TABLE 1.

I_{sc} (μA/cm^2)	V_{sc} (mV)	$F(R_o)$	E_i (mV)	R_i (kΩ.cm^2)	R_o (kΩ.cm^2)	E_{Na} (mV)
			Control			
17 ± 2·7	98 ± 5	0·81 ± 0·03	117 ± 4	1·7 ± 0·3	8·4 ± 1·6	134 ± 20
			Aldosterone/control			
2·94 ± 0·67	0·89 ± 0·05	0·83 ± 0·03	1·05 ± 0·04	0·92 ± 0·14	0·41 ± 0·09	1·10 ± 0·15

I_{sc}, short-circuit current; V_{sc}, intracellular electrical potential (V) under short-circuit conditions; $F(R_o)$, fractional resistance of the apical (0) cell border, estimated from $\Delta V_o/\Delta V_t$ upon brief transepithelial voltage clamping at 10 mV; E_i, effective e.m.f. of the basal-lateral (i) border at zero sodium transport – achieved by adding amiloride, 10^{-4} M on the outside, or by withdrawing Na from the outside; R_i, electrical resistance of the basal-lateral border, calculated from $\Delta V_{sc}/\Delta I_{sc}$ upon bringing I_{sc} to (near) zero as indicated above; R_o, electrical resistance of the apical border, obtained from $F(R_o)$ and transcellular resistance R_c which is equal to the reciprocal of difference between transepithelial and shunt (paracellular) conductance; E_{Na}, effective e.m.f. of transepithelial sodium transport (see Nagel, 1978).

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Hormonal influences on the driving force for sodium transport in amphibian epithelia

BY V. BEAUJEAN, R. BEAUWENS and J. CRABBÉ. *Department of Physiology, University of Louvain (U.C.L.) Medical School, Brussels, Belgium*

Caplan & Essig (1977) recently proposed analysis of sodium transport based on non-equilibrium thermodynamics; they suggested that the negative Gibbs free energy change, or affinity, related to the metabolic reaction driving sodium transport can be estimated experimentally (this reaction corresponds supposedly to the rate of hydrolysis of ATP at the pumping sites). According to them, affinity (A) is given by the rate of sodium transport (J_{Na}) in the absence of electrochemical gradient, divided by the slope of the relationship between the rate of oxygen consumption (J_r) and an imposed electrical potential difference ($\Delta\psi$) across the structure under study. Thus $A = -J_{Na} \times (\partial J_r/\partial \Delta\psi)^{-1}$; it is expressed in kcal per mole of oxygen consumed.

The consequences of agents credited with a definite influence on the apical membrane of the ventral skin of toad (*Bufo marinus*) and frog (*Rana temporaria*) were examined from the standpoint discussed; they were compared with the effects of prolonged (overnight) treatment of amphibian skin with aldosterone *in vitro*. Sodium transport was estimated from short-circuit current measurements.

(1) With amiloride, at a concentration of 0·5 μM on the outside, the rate of sodium transport by fresh toad skin was reduced by a half, presumably as a result of proportional reduction of sodium entry at the apical border (Sudou & Hoshi, 1977).

The drug failed to modify affinity since the latter averaged 127 ± 31 (S.E.) before amiloride and 114 ± 20 in its presence ($n = 5$; $P > 0{\cdot}2$).

(2) Vasopressin (0·1 u./ml.) raised sodium transport by fresh frog skin to a new stable level (from 25 to 43 μA/cm^2). This hormone is thought to facilitate sodium movement at the apical pole of target cells (Nagel, 1978). Affinity was uninfluenced, averaging 136 ± 27 before hormonal treatment, and 149 ± 29 after ($n = 6$; $P > 0{\cdot}2$).

(3) The influence of aldosterone (S) was evaluated by means of paired pieces of abdominal toad skin incubated overnight in Ringer fluid (NaCl, 115 mM; $KHCO_3$, 2·5 mM; $CaCl_2$, 1 mM); one piece served as a control (C) while the matched one was exposed to aldosterone, 50 nM. Sodium transport averaged 8 μA/cm^2 for C, and 22 μA/cm^2 for S; the corresponding mean affinities were 51 ± 18 and 144 ± 16 ($n = 6$; $P < 0{\cdot}01$). It is of interest that aldosterone enabled the preparations to operate as in the fresh state (cf. pre-treatment affinity value in (1) and (2)).

The number of sodium ions transported per mole of oxygen consumed was, for matched control $9{\cdot}8 \pm 0{\cdot}7$, and aldosterone-treated toad skin $10{\cdot}1 \pm 0{\cdot}5$. Sodium transport averaged 12 and 30 μA/cm^2 respectively in Ringer fluid; its rate was modified by partial substitution of magnesium for sodium in the outer solution.

Thus the degree of coupling between sodium transport and its supportive metabolism is not constant.

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Mechanical properties of isolated fish red and white muscle fibres

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Red and white muscle fibres in advanced teleosts are multiply innervated (Bone, 1964). Only white fibres generate action potentials. The responses of isolated fibre bundles from adductor operculi muscles of *Tilapia mossambica* have been investigated. Single or multiple stimuli (0·5 msec; 5 V; 1–500 Hz) were delivered at 2 min intervals and tension recorded using a silicon-beam transducer. Force–velocity (P–V) curves were obtained and Hill's constants (a/P_0, b/l_0) estimated graphically from linearly transformed data (Fig. 1*A*, inset). Maximum velocity of shortening (V_{max}), was determined by measuring the delay (ΔT) to tension redevelopment following length 'steps' (5 msec) of different amplitudes (ΔL; Fig. 1*C*).

White fibres responded to single stimuli; red fibres only responded at frequencies > 5–10 Hz. Both fibres types produced *graded*, *fused* tetani (Fig. 1*B*), reaching a maximum at 250–300 Hz. dP/dt and V_{max} (Fig. 1*A*) were greater for white than red fibres (6·5 × and 1·7 ×). The contractile responses are dependent upon extracellular Ca^{2+}; tension declined by 75 % after 2 min in Ca^{2+} deficient solutions. This effect is reversible for red fibres but only partially so for white fibres.

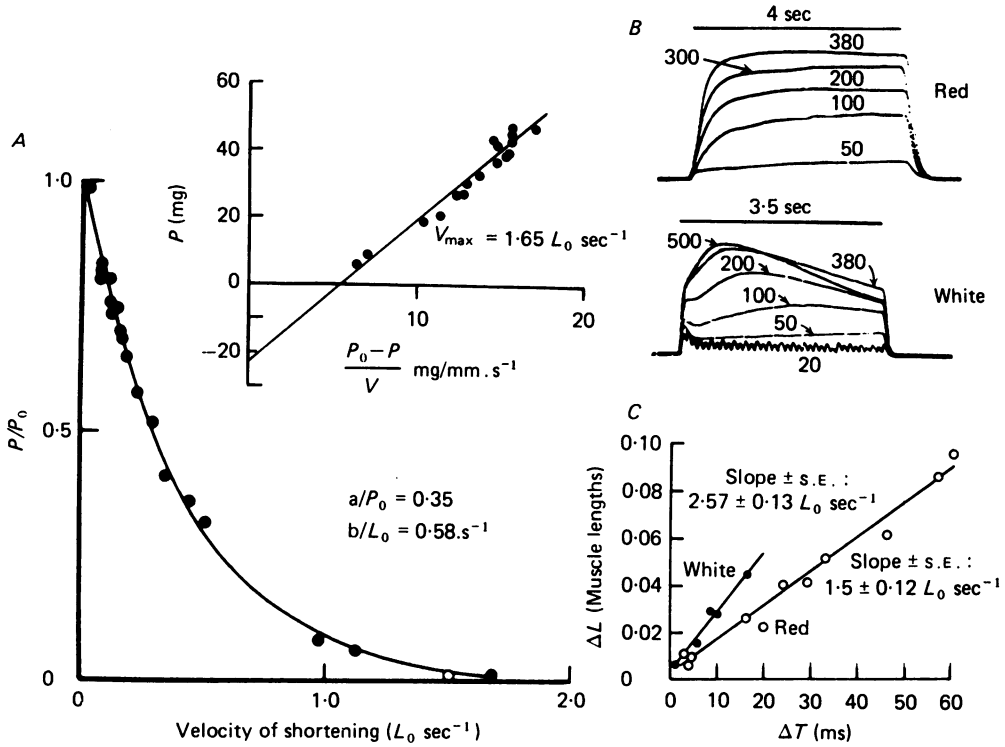

Fig. 1. *A*, force–velocity curve, red fibres; inset: P *vs.* $(P_0-P)/V$. *B*, tetani of red (*a*) and white (*b*) fibres at different stimulation frequencies. *C*, V_{max} determination. Temperature 18 °C.

Functionally, red fibres resemble amphibian tonic fibres. White fibres differ from frog twitch fibres in producing graded tetani and in their requirement for extracellular Ca^{2+}.

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Appearance of ATP in the coronary sinus effluent from isolated working rat heart in response to hypoxia

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The appearance of ATP in the coronary sinus effluent from the non-working Langendorff heart preparation in response to hypoxia was first described by Paddle & Burnstock (1974). Recently it was shown that isolated adult heart cells release ATP when exposed to brief periods of hypoxia (Forrester & Williams, 1977). The present work demonstrates the release of ATP into the coronary sinus effluent from the working heart, a preparation which approximates more closely to *in situ* conditions (Neely, Liebermeister, Battersby & Morgan, 1967).